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(54) Title: CHARACTERIZED BRCA1 AND BRCA2 PROTEINS AND SCREENING AND THERAPEUTIC METHODS BASED ON CHARACTERIZED BRCA1 AND BRCA2 PROTEINS (57) Abstract <p>Genetic analysis of familial breast and ovarian cancer indicates that BRCA1 is a tumor suppressor gene. The BRCA1 gene encodes a 190 kDa protein with sequence homology and biochemical analogy to the granin family of proteins. Granins are secreted from endocrine cells via the regulated secretory pathway and are proteolytically cleaved to yield biologically active peptides. BRCA1 protein localises to secretory vesicles, and was demonstrated to be secreted. Gene transfer of BRCA1 inhibits growth and tumorigenesis of breast and ovarian cancer cells, but not colon or lung cancer cells or fibroblasts, suggesting that BRCA1 encodes a tissue-specific growth inhibitor. Thus, BRCA1 is a secreted growth inhibitor and functions by a mechanism not previously described for tumor suppressor genes. The BRCA2 breast and ovarian cancer gene encodes a protein that also includes a granin region, indicating that the BRCA2 protein is also a secreted tumor suppressor. Therapeutic methods using the BRCA1 and BRCA2 proteins and genes are also described. A method of screening for the receptors of the BRCA1 protein and BRCA2 proteins is also described.</p>		

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DESCRIPTIONCHARACTERIZED BRCA1 AND BRCA2 PROTEINS AND SCREENING
AND THERAPEUTIC METHODS BASED ON CHARACTERIZED
BRCA1 AND BRCA2 PROTEINS

5

TECHNICAL FIELD

The present invention relates generally to purified and isolated proteins and DNA molecules; to methods of screening for receptors; and to methods of treatment of ovarian and breast cancer, and more particularly to a purified and isolated BRCA1 protein cleavage products; and to gene therapy methods using the BRCA1 gene and the BRCA2 gene in the treatment of breast and ovarian cancer; and to methods for identifying the receptors of the BRCA1 protein and the BRCA2 protein.

15

BACKGROUND OF THE INVENTION

The human breast and ovarian cancer susceptibility gene BRCA1 is mutated in the germline and lost in tumor tissue in hereditary breast and ovarian cancer (Hall et al., 1990, *Science* 250, 1684-1689; Miki et al., 1995 *Science* 266, 66-71; Smith et al., 1992; Cornelius et al., 1995, *The Breast Cancer Linkage Consortium. Genes Chrom Cancer* 13: 203-210).

20

Despite much excitement with the discovery of BRCA1, mutations were only found in the germline which accounts for only a small minority of breast cancers (Futreal et al., 1994, *Science* 266, 120-121). In addition, BRCA1 was found to be expressed at the same levels in normal individuals and sporadic breast cancers (Miki et al., 1994, *Science* 266, 66-71). Thus, the initial excitement over BRCA1 was followed by great disappointment.

25

The BRCA2 breast and ovarian cancer susceptibility gene has also recently been identified. (Wooster, R., et al., *Nature* 379: 789-792, 1995).

30

To date all tumor suppressors discovered encode proteins which are not secreted. Steeg, (review article), 1996, *Nature Genetics* 12:223. To treat the cancer associated with these tumor suppressors requires expressing the normal protein in the affected cell. Thus, these cancers have not been treatable with extracellular administration of the normal protein encoded by the tumor suppressor gene. For this reason, gene therapy has been proposed as the most likely means to supply a normal functional tumor suppressor protein.

35

This invention significantly modifies the state of the BRCA art by providing that the BRCA's are secreted and thus are amenable to direct therapy or prevention by contacting the BRCA receptor on the cell surface. In addition, the invention provides that BRCA1 is indeed underexpressed in sporadic breast cancer and thus sporadic breast cancer is amendable to therapy and prevention by correcting the BRCA deficiency. Other embodiments are also provided.

DISCLOSURE OF THE INVENTION

Both the BRCA1 and BRCA2 proteins have been identified as inhibitors of the growth of breast and ovarian cancer cells and thus a DNA segment encoding the BRCA1 protein and a DNA segment encoding the BRCA2 protein can be used in a gene therapy methods for the treatment of breast cancer and for the treatment of ovarian cancer.

The discovery and purification of the BRCA1 protein has broad utility. The purified BRCA1 protein can be used in treating breast or ovarian cancer. Moreover, since it has been determined that the BRCA1 protein is secreted, the BRCA1 protein can be also be used to identify the BRCA1 receptor. Once the BRCA1 receptor is identified, BRCA1 protein-mimetic agents which act on the receptor can be identified. Such agents are also useful in the treatment of breast and ovarian cancer.

The BRCA2 protein is also a secreted protein and can be used to identify the BRCA2 receptor. Once the BRCA2 receptor is identified, BRCA2 protein-mimetic agents which act on the receptor can be identified. Such agents are also useful in the treatment of breast and ovarian cancer.

The BRCA1 gene product is an inhibitor of the growth and proliferation of human breast and ovarian cancer cells. The BRCA1 gene product is a secreted protein, thus indicating that it acts on a receptor to produce this activity.

The BRCA2 protein is an inhibitor of the growth and proliferation of human breast and ovarian cancer cells. The BRCA2 protein is a secreted protein, thus indicating that it acts on a receptor to produce this activity.

An aspect of this invention concerns a purified and isolated BRCA1 cleavage protein; and biologically functional and structural equivalents thereof.

Another aspect of this invention is that the BRCA1 protein is a secreted tumor suppressor/growth inhibitor protein that exhibits tissue-specific tumor suppression/growth inhibition activity.

5 Important aspects of the present invention concern isolated DNA segments and recombinant vectors encoding the BRCA1 and the BRCA2 proteins, and the creation and use of recombinant host cells through the application of DNA technology, which express the BRCA1 and BRCA2 proteins.

10 The present invention concerns DNA segments, isolatable from human breast and ovarian tissue, which are free from genomic DNA and which are capable of conferring tumor suppressor/growth inhibitor activity in a recombinant host cell when incorporated into the recombinant host cell. As used herein, the term "breast or ovarian tissue" refers to normal and cancerous ovarian breast tissues, as exemplified, but not limited to, by HMEC or MCF-7 cell lines. DNA segments capable of conferring tumor suppressor activity may
15 encode complete BRCA1 and BRCA2 proteins, cleavage products and biologically actively functional domains thereof.

As used herein, the term "DNA segment" refers to a DNA molecule which has been isolated free of total genomic DNA of a particular species. Furthermore, a DNA segment encoding a BRCA1 protein or encoding a
20 BRCA2 protein refers to a DNA segment which contains BRCA1 coding sequences or contains BRCA2 coding sequences, yet is isolated away from, or purified free from, total genomic DNA of Homo sapiens. Included within the term "DNA segment", are DNA segments and smaller fragments of such segments, and also recombinant vectors, including, for example, plasmids,
25 cosmids, phage, viruses, and the like.

Similarly, a DNA segment comprising an isolated or purified BRCA1 gene or BRCA2 gene refers to a DNA segment including BRCA1 coding sequences isolated substantially away from other naturally occurring genes or protein encoding sequences or including BRCA2 coding sequences isolated
30 substantially away from other naturally occurring genes or protein encoding sequences. In this respect, the term "gene" is used for simplicity to refer to a functional protein, polypeptide or peptide encoding unit. As will be understood by those in the art, this functional term includes both genomic sequences and cDNA sequences. "Isolated substantially away from other
35 coding sequences" means that the gene of interest, in this case, the BRCA1 gene or the BRCA2 gene, forms the significant part of the coding region of the

DNA segment, and that the DNA segment does not contain large portions of naturally-occurring coding DNA, such as large chromosomal fragments or other functional genes or cDNA coding regions. Of course, this refers to the DNA segment as originally isolated, and does not exclude genes or coding regions later added to the segment by the hand of man.

In particular embodiments, the invention concerns isolated DNA segments and recombinant vectors incorporating DNA sequences which encode a BRCA1 protein that includes within its amino acid sequence the amino acid sequence of SEQ ID NO:2. In other particular embodiments, the invention concerns isolated DNA segments and recombinant vectors incorporating DNA sequences which encode a protein that includes within its amino acid sequence the amino acid sequence of the BRCA1 protein corresponding to human breast or ovarian tissue.

In particular embodiments, the invention concerns isolated DNA segments and recombinant vectors incorporating DNA sequences which encode a BRCA2 protein that includes within its amino acid sequence the amino acid sequence of SEQ ID NO:4. In other particular embodiments, the invention concerns isolated DNA segments and recombinant vectors incorporating DNA sequences which encode a protein that includes within its amino acid sequence the amino acid sequence of the BRCA2 protein corresponding to human breast or ovarian tissue.

It will also be understood that this invention is not limited to the particular nucleic acid and amino acid sequences of SEQ ID NOS: 1, 2, 3 and 4. Recombinant vectors and isolated DNA segments may therefore variously include the BRCA1 and BRCA2 encoding regions themselves, coding regions bearing selected alterations or modifications in the basic coding region, or they may encode larger polypeptides which nevertheless include BRCA1 or BRCA2 encoding regions or may encode biologically functional equivalent proteins or peptides which have variant amino acid sequences.

In certain embodiments, the invention concerns isolated DNA segments and recombinant vectors which encode a protein or peptide that includes within its amino acid sequence an amino acid sequence essentially as set forth in SEQ ID NO:2 or SEQ ID NO:4, and methods of treating breast or ovarian cancer using these DNA segments. Naturally, where the DNA segment or vector encodes a full length BRCA1 or BRCA2 protein, or is intended for use in expressing the BRCA1 or BRCA2 protein, the most preferred sequences are those which are essentially as set forth in SEQ ID NO:1 and SEQ ID NO:3

and which encode a protein that exhibits tumor suppressor activity in human breast and ovarian cancer cells, as may be determined by the breast and ovarian cancer cell growth inhibition experiments, as disclosed herein.

5 The term "a sequence essentially as set forth in SEQ ID NO:2" means that the sequence substantially corresponds to a portion of SEQ ID NO:2 and has relatively few amino acids which are not identical to, or a biologically functional equivalent of, the amino acids of SEQ ID NO:2. The term "biologically functional equivalent" is well understood in the art and is further defined in detail herein. Accordingly, sequences which have between about
10 70% and about 80%; or more preferably, between about 81% and about 90%; or even more preferably, between about 91% and about 99%; of amino acids which are identical or functionally equivalent to the amino acids of SEQ ID NO:2 will be sequences which are "essentially as set forth in SEQ ID NO:2". The term "a sequence essentially as set forth in SEQ ID NO:4" has a similar
15 meaning.

In particular embodiments, the invention concerns gene therapy methods that use isolated DNA segments and recombinant vectors incorporating DNA sequences which encode a protein that includes within its amino acid sequence an amino acid sequence in accordance with SEQ ID NO:2
20 or in accordance with SEQ ID NO:4, SEQ ID NO:2 and SEQ ID NO:4 derived from breast or ovarian tissue from Homo sapiens. In other particular embodiments, the invention concerns isolated DNA sequences and recombinant DNA vectors incorporating DNA sequences which encode a protein that includes within its amino acid sequence the amino acid sequence of
25 the BRCA1 protein from human breast or ovarian tissue, or which encode a protein that includes within its amino acid sequence the amino acid sequence of the BRCA2 protein from human breast or ovarian tissue.

In certain other embodiments, the invention concerns isolated DNA segments and recombinant vectors that include within their sequence a nucleic acid sequence essentially as set forth in SEQ ID NO:1, or a nucleic acid
30 sequence essentially as set forth in SEQ ID NO:3, and methods of treating breast or ovarian cancer using these sequences. The term "essentially as set forth in SEQ ID NO:1" is used in the same sense as described above and means that the nucleic acid sequence substantially corresponds to a portion of
35 SEQ ID NO:1, respectively, and has relatively few codons which are not identical, or functionally equivalent, to the codons of SEQ ID NO:1, respectively. Again, DNA segments which encode proteins exhibiting tumor

5 suppression activity of the BRCA1 and BRCA2 proteins will be most preferred. The term "functionally equivalent codon" is used herein to refer to codons that encode the same amino acid, such as the six codons for arginine or serine, and also refers to codons that encode biologically equivalent amino acids (see Fig. 2). The term "essentially as set forth in SEQ ID NO:3" has a similar meaning.

10 The nucleic acid segments of the present invention, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, nucleic acid fragments
15 may be prepared which include a short stretch complementary to SEQ ID NO:1 or SEQ ID NO:3, such as about 10 nucleotides, and which are up to 10,000 or 5,000 base pairs in length, with segments of 3,000 being preferred in certain cases. DNA segments with total lengths of about 1,000, 500, 200, 100 and about 50 base pairs in length are also contemplated to be useful.

20 The DNA segments of the present invention encompass biologically functional equivalent BRCA1 and BRCA2 proteins and peptides. Such sequences may arise as a consequence of codon redundancy and functional equivalency which are known to occur naturally within nucleic acid sequences and the proteins thus encoded. Alternatively, functionally equivalent proteins or peptides may be created via the application of recombinant DNA
25 technology, in which changes in the protein structure may be engineered, based on considerations of the properties of the amino acids being exchanged. Changes designed by man may be introduced through the application of site-directed mutagenesis techniques, e.g., to introduce improvements to the antigenicity of the protein or to test BRCA1 and BRCA2 mutants in order to
30 examine tumor suppression activity at the molecular level.

If desired, one may also prepare fusion proteins and peptides, e.g., where the BRCA1 or BRCA2 coding regions are aligned within the same expression unit with other proteins or peptides having desired functions, such as for purification or immunodetection purposes (e.g., proteins which may be
35 purified by affinity chromatography and enzyme label coding regions, respectively).

Recombinant vectors form important further aspects of the present invention. Particularly useful vectors are contemplated to be those vectors in which the coding portion of the DNA segment is positioned under the control of a promoter. The promoter may be in the form of the promoter which is naturally associated with the BRCA1 or BRCA2 gene(s), e.g., in breast or ovarian cancer cells, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment or exon, for example, using recombinant cloning and/or PCR technology, in connection with the compositions disclosed herein.

In other embodiments, it is contemplated that certain advantages will be gained by positioning the coding DNA segment under the control of a recombinant, or heterologous, promoter. As used herein, a recombinant or heterologous promoter is intended to refer to a promoter that is not normally associated with a BRCA1 or BRCA2 gene in its natural environment. Such promoters may include promoters isolated from bacterial, viral, eukaryotic, or mammalian cells. Naturally, it will be important to employ a promoter that effectively directs the expression of the DNA segment in the cell type chosen for expression. The use of promoter and cell type combinations for protein expression is generally known to those of skill in the art of molecular biology, for example, see Sambrook et al., 1989, *Molecular Cloning Laboratory Manual, 2d Edition*. The promoters employed may be constitutive, or inducible, and can be used under the appropriate conditions to direct high level expression of the introduced DNA segment, such as is advantageous in the large-scale production of recombinant proteins or peptides. Appropriate promoter systems contemplated for use in high-level expression include, but are not limited to, a breast selective MMTV promoter and the LXSX promoter, which are more fully described below.

As mentioned above, in connection with expression embodiments to prepare recombinant BRCA1 and BRCA2 proteins and peptides, it is contemplated that longer DNA segments will most often be used, with DNA segments encoding the entire BRCA1 or BRCA2 protein, functional domains or cleavage products thereof, being most preferred. However, it will be appreciated that the use of shorter DNA segments to direct the expression of BRCA1 and BRCA2 peptides or epitopic core regions, such as may be used to generate anti-BRCA1 or anti-BRCA2 antibodies, also falls within the scope of the invention.

DNA segments which encode peptide antigens from about 15 to about 50 amino acids in length, or more preferably, from about 15 to about 30 amino acids in length are contemplated to be particularly useful. DNA segments encoding peptides will generally have a minimum coding length in the order of about 45 to about 150, or to about 90 nucleotides. DNA segments encoding full length proteins may have a minimum coding length on the order of about 5,600 nucleotides for a protein in accordance with SEQ ID NO:2 or a minimum coding length on the order of about 10,300 nucleotides for a protein in accordance with SEQ ID NO:4.

Naturally, the present invention also encompasses DNA segments which are complementary, or essentially complementary, to the sequence set forth in SEQ ID NO:1 or the sequence set forth in SEQ ID NO:4. Nucleic acid sequences which are "complementary" are those which are capable of base-pairing according to the standard Watson-Crick complementarity rules. As used herein, the term "complementary sequences" means nucleic acid sequences which are substantially complementary, as may be assessed by the same nucleotide comparison set forth above, or as defined as being capable of base pairing to codons that encode the same amino acid, such as the six codons for arginine or serine, and also refers to codons that encode biologically equivalent amino acids (See Fig. 2).

It will also be understood that amino acid and nucleic acid sequences may include additional residues, such as additional N- or C-terminal amino acids or 5' or 3' sequences, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological protein activity where protein expression is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences which may, for example, include various non-coding sequences flanking either of the 5' or 3' portions of the coding region or may include various internal sequences, i.e., introns, which are known to occur within genes.

Excepting intronic or flanking regions, and allowing for the degeneracy of the genetic code, sequences which have between about 20% and about 50%; or more preferably, between about 50% and about 70%; or even more preferably, between about 70% and about 99%; of nucleotides which are identical to the nucleotides of SEQ ID NO:1 or to the nucleotides of SEQ ID NO:3, will be sequences which are "essentially as set forth in SEQ ID NO:1" and will be sequences which are "essentially as set forth in SEQ ID NO:3".

Sequences which are essentially the same as those set forth in SEQ ID NO:1 or as those set forth in SEQ ID NO:3 may also be functionally defined as sequences which are capable of hybridizing to a nucleic acid segment containing the complement of SEQ ID NO:1 or to a nucleic acid segment containing the complement of SEQ ID NO:3 under relatively stringent conditions. Suitable relatively stringent hybridization conditions will be well known to those of skill in the art (Sambrook et al, 1989, *Molecular Cloning Laboratory Manual, 2d Edition*).

10 List of Abbreviations

MCF-7	An immortalized cell line derived from a metastasis of human breast cancer
HMEC	A primary (non-immortalized) cell line derived from breast epithelial cells obtained during reduction mammoplasty
MDA-MB-468	An immortalized cell line derived from a metastasis of human breast cancer
Sf9	Insect cells widely used in the art with baculovirus vectors
cDNA	Complementary DNA obtained from an RNA template
DNA	Deoxyribonucleic Acid
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction

25 Figure 1 lists the C-terminal and N-terminal amino acid sequences [SEQ ID NOs:5, 6, 7] used as antigens to generate antibodies for the purified and isolated BRCA1 protein described herein.

Figure 2 is a table of the genetic code.

30 Figure 3 is a diagram showing structural features of the human BRCA1 protein [SEQ ID NO:2] covering 1864 amino acids.

Figure 4 is a diagram showing sequence alignment of the granin region of selected granin family members compared with BRCA1.

Figure 5 is a diagram showing sequence alignment of the granin region of selected granin family members compared with BRCA1 and BRCA2.

35 Figure 6 is Table I, which shows inherited BRCA1 mutations and type of cancer.

Figure 7 is Table II, which shows effect of BRCA1 Expression Vectors on growth.

Figure 8 is Table III, which shows inhibition of tumorigenesis by BRCA1.

5 Figure 9 is the sequence of the BRCA1 gene [SEQ ID NO:1].

Figure 10 is the sequence of the BRCA2 gene [SEQ ID NO:3].

Figure 11 is the sequence of the BRCA2 protein [SEQ ID NO:4].

10 Figure 12 is an immunoblot analysis of spleen and HMEC cell whole cell lysates probed with preimmune, immune, and immune plus peptide for C-19 antisera and C-20 affinity purified antibody and antibody plus peptide.

Figure 13 is an immunoprecipitation/immunoblot analysis of MDA-MB-468 cell lysates with C-19 antisera.

15 Figure 14 is a C-20 immunoblot analysis of recombinant Baculovirus produced BRCA1 (marked by arrow) compared with uninfected Sf9 cells (Control).

Figure 15 is a V8 Protease Map of Native and Recombinant BRCA1.

Figure 16 is a Pulse-Chase Analysis of MDA-MB-468 Cells.

20 Figure 17 is an immunoblot analysis of nuclear, cytoplasmic and membrane fractions of HMEC cells paired with corresponding whole cell lysate and probed for BRCA1 (C-19), c-myc, and PDGFR beta.

Figure 18 is an immunoblot analysis of nuclear, cytoplasmic and membrane fractions of HMEC cells paired with corresponding whole cell lysate and probed with D-20 N-terminal antibody plus and minus peptide.

25 Figure 19 is an immunoblot analysis of nuclear, cytoplasmic and membrane fractions of MDA-MB-468 cells paired with corresponding whole cell lysate probed with C-20 antibody.

Figure 20 depicts assay of MDA-MB-468 cell fractions produced by sucrose gradient for synaptophysin and BRCA1 immunoreactivity.

Figure 21 depicts estrogen regulation of BRCA1 protein.

30 Figure 22 depicts N-Linked glycosylation of BRCA1 protein.

Figure 23 depicts heat solubility of BRCA1 protein.

35 Figure 24 is a Western blot of HMEC cell lysates: control; stimulated with 10 mM forskolin 0.5 hours post stimulation; and 48 hours post stimulation and also includes radioimmunoprecipitation of BRCA1 From conditioned media (lane 4).

BEST MODE FOR CARRYING OUT THE INVENTION

For the purposes of the subsequent description, the following definitions will be used:

5 Nucleic acid sequences which are "complementary" are those which are capable of base-pairing according to the standard Watson-Crick complementarity rules. That is, that the larger purines will always base pair with the smaller pyrimidines to form only combinations of Guanine paired with Cytosine (G:C) and Adenine paired with either Thymine (A:T) in the case
10 of DNA or Adenine paired with Uracil (A:U) in the case of RNA.

"Hybridization techniques" refer to molecular biological techniques which involve the binding or hybridization of a probe to complementary sequences in a polynucleotide. Included among these techniques are northern blot analysis, southern blot analysis, nuclease protection assay, etc.

15 "Hybridization" and "binding" in the context of probes and denatured DNA are used interchangeably. Probes which are hybridized or bound to denatured DNA are aggregated to complementary sequences in the polynucleotide. Whether or not a particular probe remains aggregated with the polynucleotide depends on the degree of complementarity, the length of the
20 probe, and the stringency of the binding conditions. The higher the stringency, the higher must be the degree of complementarity and/or the longer the probe.

"Probe" refers to an oligonucleotide or short fragment of DNA designed to be sufficiently complementary to a sequence in a denatured nucleic acid to be probed and to be bound under selected stringency conditions.
25

"Label" refers to a modification to the probe nucleic acid that enables the experimenter to identify the labeled nucleic acid in the presence of unlabeled nucleic acid. Most commonly, this is the replacement of one or more atoms with radioactive isotopes. However, other labels include
30 covalently attached chromophores, fluorescent moieties, enzymes, antigens, groups with specific reactivity, chemiluminescent moieties, and electrochemically detectable moieties, etc.

"Tissuermizer" describes a tissue homogenization probe.

35 "PCR technique" describes a method of gene amplification which involves sequenced-based hybridization of primers to specific genes within a DNA sample (or library) and subsequent amplification involving multiple

rounds of annealing, elongation and denaturation using a heat-stable DNA polymerase.

"RT-PCR" is an abbreviation for reverse transcriptase-polymerase chain reaction. Subjecting mRNA to the reverse transcriptase enzyme results in the production of cDNA which is complementary to the base sequences of the mRNA. Large amounts of selected cDNA can then be produced by means of the polymerase chain reaction which relies on the action of heat-stable DNA polymerase produced by Thermus aquaticus for its amplification action.

"Nuclease protection assay" refers to a method of RNA quantitation which employs strand specific nucleases to identify specific RNAs by detection of duplexes.

"In situ hybridization of RNA" refers to the use of labeled DNA probes employed in conjunction with histological sections on which RNA is present and with which the labeled probe can hybridize allowing an investigator to visualize the location of the specific RNA within the cell.

"Cloning" describes separation and isolation of single genes.

"Sequencing" describes the determination of the specific order of nucleic acids in a gene or polynucleotide.

The term "BRCA1 targeted growth inhibitor agent", as used herein and in the claims, is defined as the BRCA1 protein characterized herein, whether isolated and purified directly from a natural source such as mammalian ovarian or breast cells, or produced using recombinant methods; the targeted growth inhibitor having the biological activity of tumor suppression and/or growth inhibition activity in mammalian breast or ovarian cancer cells and which binds the BRCA1 receptor; and the term "BRCA1 targeted growth inhibitor agent" also including biologically functional equivalents of the BRCA1 protein characterized herein, the term biologically functional equivalent defined herein to include, among others, proteins and protein fragments in which biologically functionally equivalent amino acids have been inserted and peptidomimetics.

The term "BRCA2 targeted growth inhibitor agent" is used herein as "BRCA1 targeted growth inhibitor agent" above but applies to BRCA2.

The term "homology" describes a mathematically based comparison of sequence similarities which is used to identify genes or proteins with similar functions or motifs.

The term "cleavage product" is defined as a polypeptide fragment produced from the targeted growth inhibitor described above by natural

proteolytic processes. Preferably such a cleavage product will have biological activity including, but not limited to, tumor suppression and/or growth inhibition activity in mammalian breast or ovarian cancer cells. This term also includes such polypeptide fragments when produced via recombinant techniques and also includes biological functional equivalents of such fragments, the term biologically functional equivalent defined herein to include, among others, proteins in which biologically functionally equivalent amino acids have been inserted and peptidomimetics.

The term "granin box domain" is defined as the consensus granin box domain of amino acids set forth in Figs. 3 and 5.

The term "recombinant host cell" is defined as a single cell or multiple cells within a cell line which are capable of undergoing genetic manipulation through well-known and art recognized techniques of transformation, transfection, transduction and the like. Examples of contemplated recombinant host cells include, but are not limited to, cell lines derived from normal or cancerous mammalian breast or ovarian tissue, other eukaryotic cells, and microorganisms. Specific examples of recombinant host cells described herein include Sf9 cells and HMEC cells.

The phrase "substantially identical to the carboxyl terminus of an amino acid sequence as essentially set forth in SEQ ID NO:2" is defined as an amino acid sequence including amino acids identical to the C-terminal amino acids in the amino acid sequence set forth in SEQ ID NO:2, or biologically functional equivalents of these amino acids. Preferred examples of the amino acid sequences are set forth in Fig. 1.

EXAMPLE 1

BRCA1 Encodes a 190 kDa Protein Expressed in Breast Epithelial Cells

As an initial step in the biochemical characterization of the BRCA1 gene product, antibodies were developed and the expression, localization, and function of BRCA1 protein were studied. These studies demonstrate that BRCA1 is a secreted, selectively growth inhibitory and represents a new member of the granin gene family.

To enable BRCA1 protein expression studies a polyclonal rabbit antisera was raised against a peptide from the C-terminal portion of the predicted BRCA1 protein [SEQ ID NO:2]. This peptide corresponded to the last 19 C-terminal amino acids (C-19) [SEQ ID NO:5], which is listed in Fig.

1. The results produced by this antibody, which are more fully described below, were confirmed with antibodies against peptides from the last 20 C-terminal amino acids (C-20) [SEQ ID NO:6] and from the first 20 N-terminal amino acids (D-20) [SEQ ID NO:7] of the predicted BRCA1 protein [SEQ ID NO:2]. These antibodies were purchased from Santa Cruz Biotechnology, Santa Cruz, CA, and the peptide sequences are also listed in Fig. 1. A search of the SWISS PROT protein sequence database for the N-terminal and C-terminal 20 amino acid peptides at the 60% homology level revealed no entries other than BRCA1. Initially these antisera were screened using Western blot analysis of whole cell lysates from normal human mammary epithelial cells (HMEC-Clonetics, (Stampfer et al., 1980, *Growth of Normal Human Mammary Cells in Culture*. 16, 415-425)) and normal human spleen. Spleen was chosen as a negative control because Northern analysis demonstrated no expression of BRCA1 in spleen (Miki et al., 1994, *Science* 266, 66-71). The results of the experiments with the C-terminal antibodies were obtained with an immunoblot analysis of spleen and HMEC cell whole cell lysates probed with preimmune, immune, and immune plus peptide for C-19 antisera and C-20 affinity purified antibody and antibody plus peptide (Fig. 12). An immunoreactive band that is blocked by the addition of corresponding peptide is present at 190 kDa in the HMEC cells for both the C-19 and C-20 anti-peptide antisera. Note that the C-19 blot has been probed with immune serum diluted 1:200 and that the C-20 blot has been probed with affinity purified antibody. No specific immunoreactivity is detected in the C-19 preimmune sera, and as expected no specific bands are detected in the spleen whole cell lysate by either C-19 or C-20. Several non-specific bands are present in the immune sera that do not block with the addition of peptide, but affinity purified C-20 antibody exhibits minimal non-specific cross reactivity. A minor band at approximately 70 kDa is identified, but appears to block with peptide indicating that this band represents a processed C-terminal fragment of the 190 kDa band. Similar studies were performed on antisera from three separate rabbits, raised against the C-terminal 19 peptide, and in each case, essentially similar results were seen, with some variation in the non-specific bands among individual rabbits, but all three react with a band at approximately 190 kDa that is not present in preimmune serum and is blocked with peptide.

A number of normal tissues and breast cancer cell lines were surveyed

majority of other cells tested showed very low to absent (MCF-7, MB-157, MB-361) levels of expression. To analyze the ability of the antisera to immunoprecipitate the 190 kDa protein, radiolabelled whole cell lysates from MDA-MB-468 cells were immunoprecipitated with C-20 antisera (Fig. 13).
5 The 190 kDa and 70 kDa species in the HMEC lane are blocked with the addition of peptide, but a number of non-specific bands including a 220 kDa species (Chen, et al, 1995, *Science* 270:789-791) are not blocked. Immunoprecipitation of MDA-MB-468 cells demonstrates a 190 kDa protein that is not present in the peptide addition control. In addition, the 70 kDa
10 species is immunoprecipitated with antibody and blocked by the addition of peptide. It is noted that several other bands are identified that are not blocked with peptide, in particular at 205 and 220 kDa. This indicates that despite the 207 kDa size predicted from the BRCA1 coding sequence, the 205 kDa and 220 kDa bands do not represent BRCA1. These results are consistent with the
15 185 kDa estrogen-regulated protein reported by Gudas (Gudas, et al. 1995, *Cancer Res.*, 55:4561-4565) but differ from the 220 kDa ubiquitous protein reported by Chen, particularly because the 220 kDa protein does not block with peptide.

While these results strongly suggested that the antisera was specific for
20 a 190 kDa protein present in breast epithelial cells, further experiments were performed to demonstrate that this protein corresponded to BRCA1. A concern was that the full length coding sequence for BRCA1 predicts a protein of 207 kDa molecular weight and the protein that the antisera recognized was definitely less than 200 kDa, and approximately 190 kDa.

25 Therefore to confirm that the antisera recognized BRCA1 a full length BRCA1 cDNA was constructed and cloned into the baculovirus transfer vector pAcSG2 (PharMingen). This plasmid was subsequently utilized to produce recombinant BRCA1 baculovirus by co-transfection and homologous recombination. The antisera was then tested for its ability to recognize
30 baculovirus expressed recombinant BRCA1. The results of these experiments were that the antibodies recognize a 180 kDa band in the BRCA1 recombinant virus infected cell lysates that is not present in the no infection control (Fig. 14). The recognition of this band is blocked by the addition of peptide and it is not present in the preimmune serum blot. To verify that the native 190 kDa
35 protein and the recombinant 180 kDa protein were in fact the same protein, peptide mapping of the 190 kDa band from MDA-MB-468 cells and the 180

kDa protein from BRCA1 recombinant Sf9 cell lysates was performed as described in the methods. The digests were loaded onto a 4-20% gradient SDS-PAGE gel and immunoblotted with C-20 (Fig. 16). In Fig. 15, Lanes 1 through 3 and 4 through 6 represent increasing concentrations of V8 protease.

5 The arrows at right indicate four identical sized molecular weight bands in lanes 3 and 6 that document that recombinant BRCA1 and the 190 kD band from MDA-MB-468 cells are identical proteins. This data confirmed that the antibodies are specific for BRCA1 protein. The difference in molecular weight between the recombinant and native protein is likely to be due to differences in glycosylation. These experiments demonstrate that the immunoreactive band completely blocks with peptide and is not present in control wild type virus infected lysates.

10 To characterize the 70 kDa species a pulse-chase experiment was performed that demonstrates that this band is a proteolytic fragment derived from the 190 kDa form. MDA-MB-468 cells were starved in cysteine and methionine deficient media and then pulsed with 35S labelled cysteine and methionine containing media with 3% dialyzed fetal bovine serum for three hours. The cells were then chased in L-15 media with 10% fetal bovine serum for increasing periods of time and harvested in lysis buffer. The lysates were immunoprecipitated, electrophoresed and the dried gel was autoradiographed (Fig. 16). In this experiment, it was shown that BRCA1 is initially synthesized as a 185 kDa form that is subsequently processed to a 190 kDa species. This represents glycosylation of the newly synthesized protein. Initially, no 70 kDa form is present, but co-incident with the appearance of the fully glycosylated form, the 70 kDa form appears. Subsequently, as the 190 kDa signal decreases with time post-labelling, the 70 kDa band increases in intensity. These findings indicate that the 70 kDa band is a proteolytic fragment, or cleavage product, of the 190 kDa protein. Other cleavage products were also isolated, including a 110 kDa species and a 130 kDa species.

30 Having demonstrated that the antibodies recognize BRCA1 protein, immunohistochemical analysis on formalin fixed, paraffin-embedded normal breast tissue were performed to analyze the distribution of BRCA1 within the breast. The results demonstrated that luminal epithelial cells (Page and Anderson, 1987, *Nature Genetics* 2, 128-131) within breast acini and ducts stain positively but myoepithelial cells and supporting stromal cells did not

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stain. No staining was observed when either primary antibody was deleted or peptide was added to the incubation. Staining was present diffusely throughout the cytoplasm and was not localized to the nucleus.

In summary, then, a 190 kDa protein was demonstrated to be the BRCA1 gene product by a number of independent criteria: 1) three different antibodies directed against two different regions of the predicted gene product react specifically in western blots and are blocked by appropriate peptides; 2) The C-20 antibody specifically immunoprecipitates the protein; 3) The C-20 antibody specifically recognizes the recombinant protein expressed in baculovirus; 4) Peptide mapping experiments definitely demonstrate that the 190 kDa protein recognized in MDA-MB-468 cells and the recombinant virus infected Sf9 cells are the same. Immunohistochemical studies indicate that BRCA1 protein is present in the luminal epithelial cells which are presumed be the cells of origin for the vast majority of hereditary and sporadic breast cancers.

EXAMPLE 2

BRCA1 is Predominately Localized in the Membrane Fraction of Breast Epithelial Cells

Due to the immunohistochemical studies, a series of experiments to determine more precisely the localization of BRCA1 within the cell was initiated. The first such experiment was a cell fractionation experiment designed to segregate nuclear, cytoplasmic, and membrane compartments of HMEC cells. As shown in Fig. 17, the cell fractionation analysis included immunoblot analysis of nuclear, cytoplasmic and membrane fractions of HMEC cells paired with corresponding whole cell lysate and probed for BRCA1 (C-19 antibody), c-myc, and PDGFR beta; and identical fractions as above probed with D-20 N-terminal antibody plus and minus peptide (Fig. 18). The cell fractionation analysis also included immunoblot analysis of nuclear, cytoplasmic and membrane fractions of MDA-MB-468 cells paired with corresponding whole cell lysate probed with C-20 antibody (Fig. 19). The results of this cell fractionation experiment clearly demonstrate that the 190 kDa species of BRCA1 is present and greatly enriched for in the membrane fraction of HMEC cells. Essentially no 190 kDa BRCA1 could be detected in either the nuclear or cytoplasmic fractions, although the 70 kDa protein is present in the nuclear fraction. As a control for the fractionation procedure parallel blots were probed with antisera for c-myc and platelet-derived growth factor receptor beta (PDGFR). These blots demonstrated that the nuclear

fraction is greatly enriched for the 67 and 64 kDa c-myc proteins (Alexandrova et al., 1995, *Mol.Cell.Biol.* 15:5188-5195) and the cytosolic and membrane fractions show PDGFR as expected. These results were confirmed with the antibody to the N-terminal portion of BRCA1 (D-20). This antibody detects the 190 kDa form of BRCA1 and an additional 165 kDa species in HMEC cells. Both of these bands are blocked with the addition of peptide and are present in the membrane fraction exclusively. Note that this antibody does not detect the 70 kDa species identified in the C-terminal peptide blots.

To investigate the possibility that subcellular localization of BRCA1 might be altered in malignant breast cells, fractionation studies on MDA-MB-468 cells that express high levels of BRCA1 protein were performed (Fig. 19). These studies demonstrated that in parallel with findings in HMEC cells the 190 kDa form of BRCA1 is also greatly enriched in the membrane fraction of MDA-MB-468 cells. In contrast to HMEC cells however, there appears to be a small amount of the 190 kDa species in the nuclear fraction of MDA-MB-468 cells. It is also noted that in contrast to HMEC cells, the 70 kDa species is present exclusively in the cytosolic fraction of MDA-MB-468 cells.

To further investigate the precise subcellular localization of BRCA1 confocal microscopy utilizing the affinity purified C-20 antisera was employed. These experiments indicated that the C-20 antibody exhibits diffuse granular staining that is predominately localized in the cytoplasm of HMEC cells. The nucleus and Golgi compartment were localized in these experiments, and this provided the capability to identify co-localization of BRCA1 in both the nucleus and Golgi complex. Simultaneous triple staining for the nucleus, Golgi complex and BRCA1 again demonstrated a predominant granular cytoplasmic distribution for BRCA1, with co-localization in both the nucleus and Golgi complex. These findings are in agreement with the cell fractionation studies of HMEC cells, despite the inability of those studies to detect the 190 kDa BRCA1 form in the nucleus, because the 70 kDa form was present in the nuclear fraction and would be expected to be detected by C-terminal antibody.

In summary, then, the above studies demonstrate that the majority of BRCA1 protein is non-nuclear and membrane-associated. Cell fractionation studies show the 190 kDa BRCA1 protein resides primarily in the membrane-associated fraction, but the p70 protein is localized in the nucleus of

normal breast cells and the cytoplasm of MB-486 breast cancer cells. The distinct membrane-associated and nuclear localization patterns result from the unprocessed and the 70 kDa processed form, respectively. There is definite co-localization with the 190 kDa BRCA1 protein and the Golgi marker supporting the trafficking of BRCA1 through the Golgi prior to its packaging into secretory granules.

EXAMPLE 3

BRCA1 is a Member of the Granin Family of Secretory Proteins and Localizes to Secretory Vesicles

Having identified BRCA1 as being present in the membrane fraction of breast epithelial cells and having a large granular cytoplasmic pattern of staining, a homology search of BRCA1 was performed, focusing on motifs that might explain the apparent membrane localization of BRCA1. A search on the SWISS PROT database of the MacDNAsis PRO v3.0 software package was performed and several features of biologic and functional importance were identified, as shown in Figure 3. In Figure 3, (-) and (+) mark location of charged residues and glyc shows potential N-linked glycosylation sites. RING finger and granin (amino acids 1214-1223) consensus are shown by open and closed boxes. Predicted protease cleavage sites for renin, kallikrein, thrombin, and trypsin are shown as thin lines. Regions deleted in the internal deletion mutants are shown as shaded boxes below (343-1081 and 515-1092).

The SWISS PROT search revealed that BRCA1 has homology to the granin consensus site as shown in Figure 4. In Figure 4, consensus sequence is shown in bold at the bottom. Sequences are human unless otherwise stated.

The granin motif spans amino acids 1214-1223 of BRCA1. Note that human BRCA1 completely satisfies the ten amino acid granin consensus and exhibits the other structural features of the family. The probability that BRCA1 would exhibit a perfect granin consensus by chance alone is 0.0018 (or one in 555). The rationale for this calculation is given at the bottom of Figure 4.

To investigate the hypothesis that BRCA1 behaves biochemically as a granin, the following series of experiments were executed. To document the presence of BRCA1 in secretory vesicles, cell organelles from MDA-MB-468 cells were fractionated by sucrose gradient centrifugation and the fractions were assayed for synaptophysin (a highly specific marker for secretory vesicles) and BRCA1 immunoreactivity. As seen in Fig. 20, coordinate expression of BRCA1 and synaptophysin was noted, which indicates the

co-localization of these proteins in secretory vesicles. These results document the co-localization of synaptophysin and BRCA1 in fractions expected to contain secretory vesicles.

5 Since granins have been shown to be regulated by estrogens (Fischer-Colbrie et al., 1991, *J. Neuroendocrinol.* 121, 125-130) HMEC cells were stimulated with estrogen and tamoxifen and increased expression of BRCA1 was demonstrated, as reported previously by others (Gudas, et al. 1995, *Cancer Res.*, 55:4561-4565; Marquis et al., 1995, *Nature Genetics* 11, 17-26; Lane et al., 1995, *Genes & Development* 9, 2712-2722). The dose response was consistent with estrogen regulation of BRCA1 expression. As presented in Fig. 21, cell lysates from HMEC cells treated for 24 hours with tamoxifen (TAM), indicated concentrations of estrogen (E2), or ethanol control (ETOH). Note E2 dosage effect.

15 HMEC cell membrane fractions were then treated with sequential deglycosylation enzymes (NANase II > O-Glycosidase DS > PNGase F to remove a2-3 and a2-6 N-acetylneuraminic acid, serine/threonine glycosylation (Fig. 22). N-linked glycosylation). A shift of protein following PNGase F treatment was noted, confirming N-linked glycosylation. Thus, BRCA1 exhibits N-linked glycosylation as predicted from the sequence analysis and shows little Ser/Thr glycosylation.

20 In addition, a heat stable fraction was prepared from recombinant baculovirus BRCA1 in a modification of the procedure of Thompson et al., (1992b), *Mol. Brain Res.* 12, 195-202, where cell pellets of infected Sf9 cells were sonicated, centrifuged, boiled for five minutes, and then centrifuged again. This heat soluble fraction was then analyzed by immunoblotting. BRCA1 remained soluble after boiling, which is characteristic of granins. As seen in Fig. 23, the immunoblots included cell lysates from uninfected Sf9 cells, wild-type infected cells (control), BRCA1 infected cells, HMEC cells, and heat soluble fraction of Baculovirus produced recombinant BRCA1. Recombinant BRCA1 remains soluble after boiling.

25 Additionally, HMEC cells were treated with 10 mM forskolin and a marked decrease in BRCA1 levels in whole cell lysates after 0.5 hours of treatment and a return to normal levels 48 hours later was observed. This data is consistent with forskolin stimulated release of secretory granules and subsequent replenishment. As seen in Fig. 24, the Western blot of HMEC cell lysates included: control, stimulated with 10 mM forskolin 0.5 hours post

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stimulation and 48 hours post stimulation. The Western blot also included a lane marked Media, which showed the results of radioimmunoprecipitation of 24 hour conditioned media from 35S-labelled MDA-MB-468 cells. These results indicate the presence of BRCA1 protein at 190 kDa. Media was supplemented with aprotinin, PMSF, leupeptin, and pepstatin.

To confirm that BRCA1 is in fact secreted MDA-MB-468 cells were metabolically labelled and the 190 kDa band was immunoprecipitated from a 24 hour collection of labelled conditioned media. Finally, immunogold electron microscopy was performed with C-20 antibody on MDA-MB-468 cells and it was demonstrated that BRCA1 immunoreactivity localizes to secretory vesicles. These secretory vesicles were primarily located in the apical cytoplasm and were often found at the tips of microvilli extending into the extracellular space. A vesicle actively undergoing secretion was identified. These findings confirm that BRCA1 is a member of the granin family of secretory proteins.

In summary, then, BRCA1 has a granin box which shows 100% homology to the consensus (Huttner et al., 1991, *Trends Biochem. Sci.* 16, 27-30) and has the expected number of acidic residues and predicted isoelectric point of granin family members. Additional evidence that BRCA1 is a granin includes 1) Presence in secretory vesicle fractions; 2) Induction by estradiol; 3) Glycosylation which occurs on secretory proteins as they are transported through the rough endoplasmic reticulum (Kornfeld & Kornfeld, 1985, *Annu. Rev. Biochem.* 54, 631-664); 4) Solubility of boiled protein, a biochemical feature of the granin family; 5) Release of BRCA1 protein by forskolin induction of regulated secretion; and 6) localization in secretory vesicles by immunogold electron microscopy.

As more fully described below, internal deletions which eliminate key structural elements and glycosylation sites destroy growth inhibition and tumor suppression, thus indicating that BRCA1 tumor suppression and growth inhibition are mediated through its granin-like properties.

EXAMPLE 4

Normal BRCA1 inhibits growth of breast and ovarian cancer cells

Experiments to determine whether BRCA1 could function as a growth inhibitor or tumor suppressor were performed. Analysis of BRCA1 protein levels in human breast cancer cell lines indicated that MCF-7 cells had little or

no BRCA1 protein. Analysis of MCF-7 cells for allelic loss at markers in the BRCA1 region indicates loss of at least 2 Mb including the BRCA1 region on one chromosome 17q21, and that the coding sequence of the retained BRCA1 allele was normal. Sal I linkered BRCA1 cDNA was cloned into the unique Xho I site of the retroviral vector LXSXN for transfection studies. To rule out trivial effects on localization or stability, two in-frame internal deletion mutants were constructed which eliminated much of the region of BRCA1 containing acidic residues and putative glycosylation sites (D343-1081 and D515-1092), but preserved the granin homology region. Two termination codon mutants were constructed which resulted in predicted proteins containing 1835 and 340 amino acids.

Table I shows that transfection of the LXSXN vector or the internal deletion mutants resulted in similar numbers of G418-resistant stable clones in a number of human cell lines. Transfection of LXSXN-BRCA1 into MCF-7 cells or Caov-4 ovarian cancer cells resulted in fewer clones which could not be expanded beyond 30 cells per clone. Some of these clones can be expanded in an enriched growth media containing GMSA, 10% fetal calf serum and 5 ng/ml EGF. This growth inhibitory effect of BRCA1 was confined to these cell types since fibroblast, lung cancer cells, and colon cancer cells were not growth inhibited by LXSXN-BRCA1. The 340-amino acid truncated protein did not inhibit growth of any cell line. However, the 1835 amino acid protein significantly inhibited growth of ovarian cancer cells but not breast cancer cells. This indicates that distinct mechanisms mediate growth inhibition of ovarian cancer cells and breast cancer cells and that this difference depends on the length of the truncated protein.

EXAMPLE 5

Ovarian cancer susceptibility is differentially associated with protein truncations 5' of the granin region

To determine whether the differential effects of short versus long truncated proteins on Caov-4 ovarian cancer cells were paralleled in human patients, the relative frequency of ovarian versus breast cancer among 166 patients in a series inheriting BRCA1 mutations was calculated (Table II). Mutations inherited by 19 patients were nonsense alterations leading to transcript instability and no mutant protein. Mutations inherited by 13 patients were missense alterations in the RING finger leading to complete but aberrant protein. All other mutations were protein-truncating mutations at sites

throughout the gene. The difference in ovarian and breast cancer distribution between the two groups was statistically significant: ovarian cancer formed a significantly lower proportion (2%) of the cancers in patients with mutant proteins that would include the granin motif compared to the proportion (25%) of cancers in patients with more severely truncated proteins ($X^2 = 11.12$, $P < 0.001$). This result is consistent with the observation that the site of BRCA1 mutation is associated with relative susceptibility to ovarian versus breast cancer (Gayther et al., 1995, *Nature Genet* 11: 428-433). The analysis of Gayther et al., indicated that the correlation between genotype and phenotype was better described by a "change point" in the BRCA1 sequence than by a linear trend in locale of mutation. The granin consensus motif at codons 1214-1223 is well within the confidence limit for the estimated location (codons 1235-1243) of the optimal change point in that analysis.

EXAMPLE 6

BRCA1 Inhibits Breast but not Colon Tumorigenesis

BRCA1 gene transfer into MCF-7 cells inhibits tumorigenesis employing retroviral gene transfer. Supernatants containing 5×10^7 vector particles from LXSXN and LXSXN-BRCA1 PA317 producer clones were used to transduce 5×10^7 MCF-7 cells or OK3 colon cancer cells in culture which were subsequently injected into the flanks of six nude mice for each vector. The cells were not treated with G418 before injection because prior G418 treatment inhibits tumorigenesis in this model, but southern blots have demonstrated that 70-80% of MCF-7 cells are transduced by this protocol. Four weeks after injection there were MCF-7 tumors in 5/6 LXSXN control mice but no tumors in LXSXN-BRCA1 mice. Retroviral transduction by BRCA1 had no effect on colon tumor formation (Table III, Fig. 8). Tumors ultimately developed in all of the control mice and 4/6 LXSXN-BRCA1 mice but the tumors in LXSXN-BRCA1 mice were significantly smaller (LXSXN: 569 grams \pm 60; LXSXN-BRCA1: 60 grams \pm 24) as illustrated in Table III, Fig. 8. Molecular analysis of tumor RNAs showed that the vector neo gene was present and expressed in all MCF tumors and that BRCA1 was detectable only in the four LXSXN-BRCA1 transduced tumors. Because the ex vivo transduction strategy could inhibit tumor establishment but not necessarily inhibit growth of already established tumors, whether in vivo injection of LXSXN-BRCA1 into established MCF-7 intraperitoneal tumors could inhibit the

growth rate and improve survival was tested. This experimental approach results in retroviral vector integration into 20-40% of tumor cells. The results showed that while all five of the mice given the mutant BRCA1 retrovirus died in less than two weeks, the five mice injected with LXS-N-BRCA1 survived from 15-41 days because the injection decreased the size and sequelae of the intraperitoneal tumors (Table III, Fig. 8).

The above studies were confirmed with stable transfectants expressing BRCA1. Using an enriched growth media MCF-7 transfectants containing the transferred BRCA1 gene were obtained. Although these clones grow at 1/3 the rate of mutant BRCA1 transfected clones in vitro, whether they would form tumors in nude mice was determined. Three distinct clones transfected with D343-1081 and four distinct clones transfected with BRCA1 (five mice per clone) were injected with the MCF-7 transfectants. The results show that 0/20 mice injected with BRCA1 transfectants developed tumors while 13/15 mice injected with mutant BRCA1 transfectants developed tumors, providing confirmation that BRCA1 inhibits tumorigenesis in nude mice (Table III). RT-PCR analysis demonstrated that the transfectants expressed the expected transfected BRCA1 or mutant BRCA1 mRNA.

Lactation is the most important secretory process in the breast and is defining for mammals. Indeed, the human breast is unique in that it does not fully differentiate until the first pregnancy and active lactation is followed by involution (Battersby et al., 1994, *Histopathology* 15:415-433). Thus during each lactation, cell numbers must be increased with the end of proliferation coinciding with the gain of secretory function. Following cessation of lactation the cell numbers must decrease to allow breast involution. Pairing secretion feedback with cell proliferation and growth inhibition mechanisms is reasonable and to be expected in this setting. The identification of BRCA1 as a member of the granin family of secreted proteins indicates that it functions as a novel type of tumor suppressor gene.

Analysis of BRCA1 mutations shows that near full-length proteins do not protect against breast cancer, but far less often lead to ovarian cancer (Table II). Analysis of transfection experiments shows that near full-length BRCA1 proteins do not inhibit growth of breast cancer cells but do inhibit growth of ovarian cancer cells. This indicates that the mechanism of tumor suppression by BRCA1 differs for breast versus ovarian cancer.

Pregnancy and lactation are important protective factors for breast

5 cancer. Although the epidemiologic basis of this is well-demonstrated, molecular correlates are lacking. The demonstration that BRCA1 mRNA is induced during mouse pregnancies and this work showing a secretory function for BRCA1 link a tumor suppressor gene with a epidemiologically-defined tumor suppression activity, early pregnancy.

EXAMPLE 7

Method of Screening for BRCA1 or BRCA2 Receptor

10 That BRCA1 is secreted has important implications for lactation and growth regulation of normal and malignant breast cells. The secreted BRCA1 protein acts on a cell surface receptor. The interaction between the BRCA1 protein and the receptor produces the beneficial effects, i.e. tumor suppression, in the target breast or ovarian tissue. Methods for isolating the BRCA1 receptor follow. The BRCA2 receptor can be similarly isolated.

15 Baculovirus BRCA1 can be purified from the insect cells with the C20 antibody and then labelled with radioactive iodine by standard methods. Cys61Gly and termination codon mutant BRCA1 proteins are prepared and labelled as a control. The labelled BRCA1 can then be used to perform binding studies to identify cells with BRCA1 receptors using Scatchard analysis; and to
20 perform cross-linking studies which demonstrate the BRCA1 receptor(s) on polyacrylamide gels. These initial characterization methods are used to identify cells with high and low numbers of BRCA1 receptor(s) for purification and isolation studies. Once a cell line with high levels of BRCA1 receptor has been identified, then the protein is purified by the following approaches:

25 Approach A: Biochemical purification

The cell line which expresses high levels of BRCA1 receptor is lysed and the protein from cell lysates or membrane preparations is purified by gel filtration followed by purification of the receptor with a column containing the
30 BRCA1 ligand bound to a solid phase such as sepharose. The purified receptor protein can then be microsequenced and the gene cloned using degenerate oligonucleotides derived from the protein sequence.

Approach B:

35 Ligand is radiolabeled with ¹²⁵I and then used to screen cell lines or tissues for specific binding by Scatchard analysis. Once such binding is identified, a cDNA library is constructed from that tissue or cell line and transfected into a cell line that does not exhibit specific binding. These

transfected cells are then screened for newly acquired specific binding which indicates they have been transfected with a construct containing the gene for the BRCA1 receptor. Plasmid DNA from positive clones is then isolated and sequenced for identification. This single construct is then transfected back into the null cells to verify that binding of ligand is mediated by the transfected gene. (Kluzen et al, *Proc Natl Acad Sci USA* 89:4618-4622 (1992).

Alternatively, chimeric BRCA1 and immunoglobulin Fc molecules can be constructed. (LaRochelle et al, *J Cell Biol* 129:357-366 (1995)). These chimeric molecules are then be used to screen for binding to BRCA1 receptor on whole cells via flow cytometry. Alternatively, due to the presence of the immunoglobulin component of the molecule, cell lysates are screened by immunoblotting or by immunoprecipitation of metabolically labelled cells. This technique can identify BRCA1 binding proteins by a variety of different methods. Peptide digests of the identified proteins are then generated so that the peptides can be sequenced and the whole molecule cloned by a degenerative oligonucleotide approach.

EXAMPLE 8

Screen for BRCA1 Protein Mimetic Agents

Classical methods for identifying compounds which activate receptors are greatly facilitated by the prior identification of the receptor. However, knowledge of ligand structure domains and deletion and minimization methods allow the identification of active ligand mimetic drugs without first finding the receptor. As more fully described above, certain regions of the BRCA1 gene have been deleted to show which regions are essential for growth inhibitory activity. These studies can be continued in a systematic manner, revealing the regions of the molecule needed for its key activities. Upon identification of a small protein that can produce growth inhibition, systematic structural and functional analysis of the minimal protein can be performed as per the methods described in Li, et al., *Science* 270: 1657, 1995. Drugs can then be screened for and/or synthesized which mimic the peptide structure and consequently produce the desired effect.

Thus, provided also is a method of screening a compound for tumor suppressor activity comprising contacting the compounds with the BRCA1 or BRCA2 receptor, a compound which binds the receptor indicating a compound having potential tumor suppressor activity. Binding can be detected by well-

known methods in the art, including, among others, radioimmunoassays and fluorescence assays.

Example 9

5 Therapy method for ovarian cancer using the BRCA1 Gene.

10 Viral vectors containing a DNA sequence that codes for a protein having an amino acid sequence as essentially set forth in SEQ ID NO:2 can be constructed using techniques that are well known in the art. This sequence includes the BRCA1 protein. Viral vectors containing a DNA sequence
15 essentially as set forth in SEQ ID NO:1 (the BRCA1 gene) can be also constructed using techniques that are well known in the art. Retroviral vectors such as the LXS vector described above, adenoviral vectors, or adeno-associated viral vectors are all useful methods for delivering genes into ovarian cancer cells. The viral vector is constructed by cloning the DNA sequence
20 essentially as set forth in SEQ ID:1 into a retroviral vector such as an ovarian selective vector. Most preferably, the full-length (coding region) cDNA for BRCA1 is cloned into the retroviral vector. The retroviral vector would then be transfected into virus producing cells in the following manner: Viruses are prepared by transfecting PA317 cells with retroviral vector DNAs which are purified as described in Wong et al., 1988, *Proceeding of the UCLA Symposia on Biology of Leukemias and Lymphomas.*, Golde D. (ed.), Alan R. Liss, Inc. 61:553-566. Following transfection, the PA317 cells are split and then treated with G418 until individual clones can be identified and expanded. Each clone is then screened for its titer by analyzing its ability to transfer G418 resistance
25 (since the retroviral vector contains a Neomycin resistance gene). The clones which have the highest titer are then frozen in numerous aliquots and tested for sterility, presence of replication-competent retrovirus, and presence of mycoplasma. Methods generally employed for construction and production of retroviral vectors have been described above and in Miller, et al., 1990, *Methods in Enzym.* 217:581-599.
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35 Once high titer viral vector producing clones have been identified, then patients with ovarian cancer can be treated by the following protocol: Viral vector expressing BRCA1 is infused into either solid tumors or infused into malignant effusions as a means for altering the growth of the tumor (since it is shown above that the BRCA1 protein decreases the growth rate of ovarian cancer cells). Because viral vectors can efficiently transduce a high percentage

of cancer cells, the tumors will be growth inhibited.

EXAMPLE 10

The protein encoded by the BRCA2 breast and ovarian cancer susceptibility gene is a granin and a secreted tumor suppressor.

The protein encoded by the BRCA2 breast and ovarian cancer susceptibility gene (Wooster, R., et al., *Nature* 379: 789-792, 1995) includes a domain similar to the granin consensus at the C-terminus of the protein. As seen in Fig. 5, the sequence at amino acids 3334-3344 of Genbank locus HUS43746 matches six of the seven constrained sites of the granin consensus.

BRCA2 and murine BRCA1 differ from the consensus at the same site. The granin motif in BRCA2 lies at the extreme C-terminal end of the protein, a locale characteristic of a known granin. This indicates that the protein encoded by the BRCA2 gene is also a secreted growth inhibitor. Use of both the BRCA1 and BRCA2 genes offer the opportunity for a unified approach to the treatment of inherited and sporadic breast cancer. Accordingly, the examples set forth above depicting the treatment of ovarian cancer, are equally applicable to the BRCA2 gene and the BRCA2 protein.

The identification of BRCA1 and BRCA2 as granins indicated that there is a granin superfamily of which consists of the subfamilies of chromogranins (chromogranins A, B and C); secretogranins (secretogranins III-V) and the BROCAgranins (BRCA1, BRCA2 and other tumor suppressor genes). This classification of granins into these subclasses is based on greater similarities within the subfamilies than with the superfamily as a whole. For example, the chromogranins share an additional region of homology besides the granin consensus and exhibit similar expression patterns; the secretogranins show less homology to the granin consensus than either chromogranins or BROCAgranins; the BROCAgranins BRCA1 and BRCA2 are cancer susceptibility genes, contain additional regions of homology, and are significantly larger (two-twenty times larger) than other granins described to date.

Thus, the invention provides in Example 3 and in this example a granin box consensus sequence shown in Figure 5. Thus, provided is a family of proteins which share the consensus sequence that are tumor suppressor genes. BRCA1 and BRCA2 are members of this family. Other members may be identified and purified as tumor suppressor genes by genetic methods, by

DNA-based searches for granin homology; or by cloning and characterization of granins in ovarian or breast cancer cells by biochemical methods. Such biochemical methods include the isolation and purification of proteins from secretory vesicles or Golgi by physical isolation methods, followed by development of antibodies to determine which proteins, followed by cloning of genes for secreted proteins after protein sequencing and cloning with degenerate oligonucleotide primers. A example of this method is described in Colomer et al., 1996, *J. Biological Chemistry* 271:48-55. Thus, other BROCAgranins are contemplated to be within the scope of this invention.

EXAMPLE 11

Gene Therapy method using the BRCA2 Gene

Viral vectors containing a DNA sequence that codes for a protein having an amino acid sequence as essentially set forth in SEQ ID NO:4 can be constructed using techniques that are well known in the art, and as are more fully described above. This sequence includes the BRCA2 protein. Viral vectors containing a DNA sequence essentially as set forth in SEQ ID NO:3 (the BRCA2 gene) can be also constructed using techniques that are well known in the art. Retroviral vectors, adenoviral vectors, or adeno-associated viral vectors are all useful methods for delivering genes into breast cancer cells. An excellent candidate for use in breast cancer gene therapy is a Moloney-based retroviral vector with a breast selective MMTV promoter (Wong et al., 1988, *Proceeding of the UCLA Symposia on Biology of Leukemias and Lymphomas.*, Golde D. (ed.), Alan R. Liss, Inc. 61:553-566). The viral vector is constructed by cloning the DNA sequence essentially as set forth in SEQ ID NO:3 into a retroviral vector such as a breast selective vector. Most preferably, the full-length (coding region) cDNA for BRCA2 is cloned into the retroviral vector. The retroviral vector is then transfected into virus producing cells in the following manner: Viruses are prepared by transfecting PA317 cells with retroviral vector DNAs which are purified as described in Wong et al. Following transfection, the PA317 cells are split and then treated with G418 until individual clones can be identified and expanded. Each clone is then screened for its titer by analyzing its ability to transfer G418 resistance (since the retroviral vector contains a Neomycin resistance gene). The clones which have the highest titer are then frozen in numerous aliquots and tested for sterility, presence of replication-competent retrovirus, and presence of

mycoplasma. The methods generally employed for construction and production of retroviral vectors have been described above and in Miller, et al., 1990, *Methods in Enzym.* 217:581-599.

Once high titer viral vector producing clones have been identified, then patients with breast cancer can be treated by the following protocol: Viral vector expressing BRCA2 protein is infused into either solid tumors or infused into malignant effusions as a means for altering the growth of the tumor. Because viral vectors can efficiently transduce a high percentage of cancer cells, the tumors will be growth inhibited.

EXAMPLE 12

Gene Transfer Using Liposomes

An alternative method of gene therapy using the BRCA1 and BRCA2 gene includes the use of liposome to deliver the DNA into the cells. By this method, the above described LXS-N-BRCA1 plasmid would be incubated with a liposome preparation such as cationic liposomes and then the DNA liposome mix is added to cells or injected into an animal or patient. Generally, the liposome transfection method is of a lower efficiency than viral gene transfer methods. This method is useful because the BRCA1 and BRCA2 proteins are secreted proteins. Thus, if only a few percent of cells take up the DNA-liposome combination, it is likely that enough BRCA1 or BRCA2 protein will be produced and secreted from these cells to growth inhibit other cells. Liposomal transfection of nucleic acids into host cells is described in U.S. Patent Nos. 5,279,833 and 5,286,634, the contents of each of which are herein incorporated by reference.

EXAMPLE 13

Anti-Sense Inhibition of the Production of BRCA1 Protein

The antisense inhibition of BRCA1 is described as follows. Antisense methods were used to demonstrate that BRCA1 expression inhibits cell growth. Unmodified 18 base deoxyribonucleotide complementary to the BRCA1 translation initiation site were synthesized and added to cultures of primary mammary epithelial cells (Stampfer et al. 1980, *In Vitro* 16: 415-425 (1980)) or MCF-7 breast cancer cells (Soule and McGrath, 1980, *Cancer Letters* 10, 177-189 (1980)).

The morphologic appearance of the cell lines was not noticeably

changed by addition of antisense oligonucleotide, but the proliferative rate was faster. Incubation of cells with 40 uM anti-BRCA1 oligonucleotide produced accelerated growth of both normal and malignant mammary cells, but did not affect the growth of human retinal pigmented epithelial cells. An intermediate
5 dose of anti-BRCA1 oligonucleotide produced a less pronounced but significant increase in cell growth rate. This was not a toxic effect of the oligonucleotide since a control "sense" oligomer with the same GC content did not increase the proliferation rate, and because an addition of a 10 fold excess of sense oligomer to the anti-BRCA1 oligomer reversed the growth activation.

10 Thus, antisense inhibition of BRCA1 accelerates the growth of breast cancer cells. Because chemotherapy is most effective in cancer cells which are rapidly dividing, it is possible then to treat breast or ovarian cancer by accelerating growth of cancer cells by antisense inhibition of BRCA1 protein expression and by treating with chemotherapeutic drugs using standard
15 chemotherapy protocols.

Example 14

Biological Functional Equivalent Proteins and Peptides

20 Modification and changes may be made in the structure of the BRCA1 protein and the BRCA2 protein, or in cleavage products of these proteins, and still obtain a molecule having like or otherwise desirable characteristics. For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or
25 binding sites on substrate molecules or receptors, specifically the BRCA1 or BRCA2 receptor. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence (or, of course, its underlying DNA coding sequence) and nevertheless obtain a protein with like
30 (agonistic) properties. Equally, the same considerations may be employed to create a protein or polypeptide with countervailing (e.g., antagonistic) properties. It is thus contemplated by the inventors that various changes may be made in the sequence of the BRCA1 and BRCA2 proteins or peptides (or underlying DNA) without appreciable loss of their biological utility or activity.

35 Two designations for amino acids are used interchangeably throughout this application, as is common practice in the art. Alanine = Ala (A); Arginine = Arg (R); Aspartate = Asp (D); Asparagine = Asn (N); Cysteine

= Cys (C); Glutamate = Glu (E); Glutamine = Gln (Q); Glycine = Gly (G);
Histidine = His (H); Isoleucine = Ile (I); Leucine = Leu (L); Lysine = Lys
(K); Methionine = Met (M); Phenylalanine = Phe (F); Proline = Pro (P);
Serine = Ser (S); Threonine = Thr (T); Tryptophan = Trp (W); Tyrosine =
5 Tyr (Y); Valine = Val (V).

It is also well understood by the skilled artisan that, inherent in the
definition of a biologically functional equivalent protein or peptide, is the
concept that there is a limit to the number of changes that may be made within
a defined portion of the molecule and still result in a molecule with an
10 acceptable level of equivalent biological activity. Biologically functional
equivalent peptides are thus defined herein as those peptides in which certain,
not most or all, of the amino acids may be substituted. Of course, a plurality
of distinct proteins/peptides with different substitutions may easily be made
and used in accordance with this invention.

15 It is also well understood that where certain residues are shown to be
particularly important to the biological or structural properties of a protein or
peptide, e.g., residues in active sites, such residues may not generally be
exchanged. This is the case in the present invention where an exchange in the
granin box domain may alter the fact that the BRCA1 and BRCA2 proteins are
20 secreted.

Amino acid substitutions are generally based on the relative similarity
of the amino acid side-chain substituents, for example, their hydrophobicity,
hydrophilicity, charge, size, and the like. An analysis of the size, shape and
type of the amino acid side-chain substituents reveals that arginine, lysine, and
25 histidine are all positively charged residues; that alanine, glycine and serine are
all a similar size; and that phenylalanine, tryptophan and tyrosine all have a
generally similar shape. Therefore, based upon these considerations, arginine,
lysine and histidine; alanine, glycine and serine; and phenylalanine, tryptophan
and tyrosine; are defined herein as biologically functional equivalents.

30 In making such changes, the hydropathic index of amino acids may be
considered. Each amino acid has been assigned a hydropathic index on the
basis of their hydrophobicity and charge characteristics, these are: isoleucine
(+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine
(+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7);
35 serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2);
glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-
3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte & Doolittle, 1982, incorporated herein by reference). It is known that certain amino acids may be substituted for another amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, the substitution of amino acids whose hydropathic indices are within ± 1 are particularly preferred, and those within ± 2 is preferred, those which are within ± 0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biological functional equivalent protein or peptide thereby created is intended for use in immunological embodiments. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e. with a biological property of the protein. It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein.

As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 \pm 1); glutamate (+3.0 \pm 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 \pm 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4).

In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

While discussion has focused on functionally equivalent polypeptides arising from amino acid changes, it will be appreciated that these changes may be effected by alteration of the encoding DNA; taking into consideration also that the genetic code is degenerate and that two or more codons may code for the same amino acid.

Kyte & Doolittle, *J. Mol. Biol.*, 157:105-132, 1982; Hopp, U.S. Patent 4,554,101

In addition to the peptidyl compounds described herein, the inventors also contemplate that other sterically similar compounds may be formulated to mimic the key portions of the peptide structure. Such compounds, which may be termed peptidomimetics, may be used in the same manner as the peptides of the invention and hence are also functional equivalents. The generation of a structural functional equivalent may be achieved by the techniques of modelling and chemical design known to those of skill in the art. It will be understood that all such sterically similar constructs fall within the scope of the present invention.

U.S. Patent 4,554,101 (Hopp, incorporated herein by reference) teaches the identification and preparation of epitopes from primary amino acid sequences on the basis of hydrophilicity. Through identify epitopes from within an amino acid sequence such as the BRCA1 and BRCA2 sequences disclosed herein (SEQ ID NOs:2, 4). These regions are also referred to as "epitopic core regions".

Numerous scientific publications have been devoted to the prediction of secondary structure, and to the identification of epitopes, from analyses of amino acid sequences (Chou & Fasman, 1974a,b; 1978a,b 1979). Any of these may be used, if desired, to supplement the teachings of Hopp in U.S. Patent 4,554,101. Moreover, computer programs are currently available to assist with predicting antigenic portions and epitopic core regions of proteins. Examples include those programs based upon the Jameson-Wolf analysis (Jameson & Wolf, 1998; Wolf et al., 1988), the program PepPlot® (Brutlag et al., 1990; Weinberger et al., 1985), and other new programs for protein tertiary structure prediction (Fetrow & Bryant, 1993).

Example 15

Treatment of Breast or Ovarian Cancer using Purified BRCA1 or BRCA2 Protein

Alternatively, breast or ovarian cancer be treated by the administration of a therapeutically effective amount of the BRCA1 or BRCA2 protein via an efficient method, such as injection into a tumor. A therapeutically effective amount can be determined by one having ordinary skill in the art using well-known protocols.

It is important to note that breast and ovarian cancer cells have surface receptors which must be contacted by the BRCA1 or BRCA2. Thus, the BRCA1 or BRCA2 protein, an active fragment, or a small molecule mimetic binds directly to a receptor on the surface of the breast or ovarian cancer cells.

Example 16

Method of Treating Breast or Ovarian Cancer Comprising Introducing the BRCA1 Receptor Gene and the BRCA1 protein into a Breast or Ovarian Cancer Cell

The loss of the BRCA1 receptor in breast and ovarian cancer cells will lead to the proliferation and tumorigenesis in these cells. Thus, breast and ovarian cancer can be treated by introducing the BRCA1 receptor gene into breast or ovarian cancer cells using the gene therapy methods described above.

This step will be followed by the administration of a therapeutically effective amount of the BRCA 1 protein so that the BRCA1 protein contacts a receptor on a surface of the breast or ovarian cells. A therapeutically effective amount can be determined by one having ordinary skill in the art using well-known protocols.

Example 17

Method of Preventing Breast or Ovarian Cancer using BRCA1 or BRCA2 Protein

It is a well-established epidemiologic fact that parity and particularly early parity has a protective effect in regards to both breast and ovarian cancer risk. Because of various changes in the structure of society it is now quite common for women to delay childbirth and lose this natural protective effect. Since it is known that BRCA1 is induced in pregnancy and lactation, and it is demonstrated herein that BRCA1 is a secreted growth inhibitor that is specific for breast and ovarian cancer, the protective effect of pregnancy and lactation is due to BRCA1 expression. BRCA1 mediation of this effect for both breast and ovarian cancer presents a variety of strategies that are useful in decreasing breast and ovarian cancer risk, particularly in women that did not have a baby

in their first twenty years and thus, were at a higher risk to develop breast or ovarian cancer. Thus, one can use a BRCA to prevent the first occurrence or a recurrence of breast and ovarian cancer. Examples of such strategies are presented below. While examples are provided, such strategies should not be limited to the examples.

BRCA1 protein might be used a chemopreventive agent by introducing BRCA1 directly into the peritoneal cavity of women as the whole protein, as a functional fragment, or as a functional cleavage product. In addition, compounds that induce expression of BRCA1 or activate its receptor, e.g. a small molecule mimetic, could also be introduced. Since BRCA1 is a secreted protein, the introduced BRCA1 will decrease ovarian cancer risk in the same manner that BRCA1 does normally when its expression is induced by pregnancy. The protective effect is also expected where BRCA1 expression is mediated by gene therapy method by either directly or indirectly inducing expression of BRCA1.

A similar rationale can be applied to breast cancer prevention. In this case, the whole BRCA1 protein; a functional fragment or a functional cleavage product thereof; or a pharmacological mimic can be used. In addition, compounds that induce expression of BRCA1 or activate its receptor, e.g. a small molecule mimetic, could also be used. Gene therapy approaches for increasing the expression of BRCA1 in breast directly or indirectly could also be used. Systemic agents that induce expression of BRCA1, or that mimic function and can replace BRCA1, such a peptidomimetic agent, could also be used. The delivery of such agents could take place by directly instilling the agent within the breast by introducing via the nipple. Finally, an implantable time release capsule can be used in a prevention strategy, either by placing such a capsule in the peritoneum for ovarian cancer, by implant such a capsule into the breast for breast cancer.

Since the BRCA2 protein includes a granin sequences and is also a secreted tumor suppressor protein, similar prevention strategies can be applied using the BRCA2 gene and protein.

Experimental Procedures for Examples 1-6

Tissues and Cell Culture

Cryopreserved primary cell lines (Passage 7) of normal human mammary epithelial (HMEC) cells, were obtained from Clonetics, Inc. The cryovial of HMEC was thawed and subcultured according to the instructions provided,

which are a slight modification of published procedures (Stampfer et al, 1980, *Growth of Normal Human Mammary Cells in Culture*. 16, 415-425). Breast cancer cell lines were obtained from American Type Culture Collection (ATCC), Rockville, MD. Sf9 cells were obtained from ATCC.

5

Antibodies

C-terminal 19 peptide fragment was conjugated to keyhole limpet hemacyanin and injected into New Zealand white rabbits along with Freund's adjuvant according to standard protocols. C-20 and D-20 were provided by Santa Cruz Biotechnology. c-myc and PDGFR antibodies were provided by Steve Hann and William LaRochelle, respectively.

10

15

Cell Extracts, Immunoblotting, Immunoprecipitation, Northern blotting Cell lysates, immunoblotting, and immunoprecipitation assays were performed according to previously published methods (Jensen et al, 1992, *Biochem.* 31: 10887-10892). RNA was isolated by published methods (Jensen et al, 1994, *Proc Natl Acad Sci USA* 91, 9257-9261) and probed with the T7 labelled EcoRI- Kpn I fragment from exon 11.

20

Cell Fractionation Studies

25

30

35

Cell fractionations were performed according the method of Fazioli, et al (1993, *Mol. Cell. Bio.* 13, 5814-5828). Briefly, cells in T175 flasks were washed twice with cold PBS/0.5 mM sodium vanadate, followed by a single washing in cold isotonic fractionation buffer (FB). Then, cold FB + protease inhibitors (PI) are added to the plates. The plates are incubated for 10 min, scraped, and homogenized with a Dounce tissue homogenizer. The nuclei were gently pelleted (375g) at 4°C and the supernatant (cytosolic and plasma membrane fraction) was saved. After washing the nuclear pellet with four aliquots of cold FB + PI + 0.1% NP40 followed by centrifugation at 4°C, the nuclei were resuspended in cold FB and 2X lysis buffer + PI. The cytosolic and plasma membrane fraction was then ultracentrifuged (35,000g) for 30 min at 4°C and the supernatant was saved as the cytosolic fraction. The pellet (plasma membrane fraction) was resuspended in FB + PI and solubilized in 2X lysis buffer with PI. Following this, the nuclear and plasma membrane fractions are sonicated on ice for 10 seconds three times. They were then spun

at 10,000g at 4°C, and the supernatant was collected and saved as the soluble nuclear and plasma membrane fractions, respectively.

Confocal Imaging Studies

5 HMEC cells were plated into 35 mm culture dishes with glass bottom cover slips (Mat-Tek) and allowed to grow to 70% confluency. The cells were then rinsed, fixed in 4.0% paraformaldehyde in phosphate buffered saline at 4°C (PBS, 0.01 M phosphate salts, and 0.15 M NaCl, pH 7.6) for ten minutes, and washed and permeabilized in PBS with 0.2% Triton X-100 for two minutes.
10 Cells were blocked with 5% normal donkey serum in PBS. Primary antibodies were diluted in PBS containing 3.0% bovine serum albumin (BSA) and 0.1% Triton X-100 and consisted of rabbit anti-BRCA-1 (vondor) diluted 1:200 and a mouse monoclonal to a Golgi complex antigen (Biogenex; clone 371-4) diluted 1:10. No antibody and antibody to BRCA-1 pre-adsorbed with the peptide antigen were used as negative controls. Secondary antibodies were
15 from Jackson ImmunoResearch and consisted of extensively adsorbed, multiple-labeling grade donkey anti-rabbit-specific IgG conjugated to CY3 (diluted 1:1000) and donkey anti-mouse-specific IgG conjugated to either CY5 (diluted 1:500) or FITC (diluted 1:250). Nuclei were counterstained with
20 YO-PRO1 (Molecular Probes, Inc.) diluted 1:500 for 20 minutes following immunostaining. Double-immunolabeling studies were carried out with all the necessary controls for staining specificity as outlined previously (Jetton et al., 1994, *J. Biol. Chem.* 269, 3641-3654). Following immunostaining, sections were mounted in Aqua-Polymount (Polysciences) and imaged using a Zeiss
25 LSM 410 confocal microscope using the 488/647 and 543 nm lines of an Ar-Kr and He-Ne laser, respectively. Images were optimized using Adobe Photoshop 3.0 then transferred as TIFF files to a Silicon Graphics Indigo where figures were assembled using SGI Showcase and printed using a Tektronix Phaser IISDX color printer.

30

Glycosylation Analysis

Glycosylation analysis was performed on aliquots of HMEC membrane fractions with the Enzymatic Deglycosylation Kit from Glyko, Inc. according to the manufacturer's recommended protocol, and the samples were
35 immunoblotted and probed with C-20 antibody.

Isolation of Secretory Vesicles

Secretory vesicles were isolated as described (Tooze and Huttner, 1990, *Cell* 60, 837-847) with minor modifications. All steps were performed at 4°C. MDA-MB-468 cells were washed with cold PBS containing protease inhibitors. After centrifugation at 700 x g for 5 min, the pellet was resuspended in homogenization buffer (0.25 M sucrose, 1 mM EDTA, 1 mM Mg acetate, 10 mM HEPES-KOH, pH 7.2) with protease inhibitors and centrifuged at 1700 x g for 5 min. The pellet was resuspended in 5 times the cell volume of homogenization buffer with protease inhibitors. Cells were passed through a 22 gauge needle 10 times and homogenized with 50 strokes of a Pyrex homogenizer. Unbroken cells and nuclei were pelleted at 1000 x g for 10 min. One ml of the postnuclear supernatant was loaded onto a 0.3 M-1.2 M sucrose gradient (made in 10 mM HEPES-KOH, pH 7.2) with protease inhibitors and centrifuged at 25,000 rpm in a Beckman SW41 rotor for 15 min. One ml fractions were collected from the bottom and fractions 9-12 were pooled and loaded onto a 0.5 M-2 M sucrose gradient. The gradient was centrifuged at 25,000 rpm in a Beckman SW41 rotor for 16 hours and fractions collected from the bottom. Fractions 4-12 were analyzed by Western blot analysis.

Expression of Recombinant Clones in the Baculovirus Expression System

A full length BRCA1 cDNA containing consensus translation initiation and stop sites was cloned into the baculovirus transfer vector pAcSG2 as a Sal I fragment. Recombinant baculovirus were produced by cotransfecting Sf9 cells with Baculogold (PharMingen) virus DNA and the recombinant vector DNA. The resulting culture supernatants were harvested after four days, screened for homologous recombination by limiting dilution (Jensen et al., 1992, *Biochem.* 31: 10887-10892), and confirmed by dot-blot hybridization using the 32P-labeled, BRCA1 cDNA probe. Recombinant protein was expressed by infecting with high titer virus at multiplicities of infection of 10:1 or greater.

Peptide Mapping

Whole cell lysates from MDA-MB-468 cells and BRCA1 recombinant virus infected Sf9 cells were electrophoresed and the 190 kDa MDA-MB-468 band and 180 kDa BRCA1 recombinant protein were identified by removing one lane for immunoblotting with C-20 antibody. The bands of interest were then

cut out of the gel, eluted on Microcon spin columns (Amicon), and digested with increasing amounts of V8 protease. The digests were re-electrophoresed on 4-20% gradient gels and immunoblotted with C-20.

5 Immunogold electron microscopy

MDA-MB-468 cells were trypsinized, washed in PBS, and fixed in 4.0% paraformaldehyde + 0.1% glutaraldehyde/PBS (pH 7.4) for 10 minutes on ice. The cell pellet was washed in PBS, dehydrated in a graded series of alcohols, and embedded in LR White resin (medium grade; Polysciences, Inc.). Thin sections were mounted on nickel grids and blocked in PBS + 1.0% bovine serum albumin (BSA) for two hours at room temperature. The grids were then incubated overnight in 1.0% BSA supplemented with 0.05% Tween with or without the C-20 antibody at a final dilution of 1:200. The grids were then washed in PBS/0.05% Tween and incubated in a 1:100 dilution of a goat anti-rabbit-gold conjugate (15 nm size; Electron Microscopy Sciences) for one hour at room temperature. The grids were washed as above, rinsed in distilled water and lightly counterstained with saturated aqueous uranyl acetate and lead citrate, and imaged with a Hitachi H-800 transmission electron microscope.

20

Gene Transfer Methods and Nude Mice Studies

MCF-7 cells were transfected by calcium phosphate coprecipitation for cell growth studies, but were transduced with retroviral stocks from PA317 producer clones for the nude mice studies as described in the results. Cultured MCF-7 cells were transduced in vitro and then injected subcutaneously into the left flank of 4 week old female nu/nu mice containing slow-release estrogen pellets (Soule et al., 1980, *Cancer Letters* 10, 177-189). Tumor size was determined weekly and animals were autopsied at 8 weeks after injection for determination of tumor weight and RT-PCR analysis for gene expression (Thompson et al., 1995, *Nature Genetics* 9, 444-450). For evaluation of effects of BRCA1 and mutant retroviral vectors on established tumors, 10⁷ MCF-7 cells were injected intraperitoneally and the animals were injected intraperitoneally with high titer retroviral vector stock (10⁷ virions) once palpable tumors were identified.

30

Example 18Phase I Trial of Retroviral BRCA1 Gene Therapy in Ovarian Cancer

5

Summary

Methods. As an initial step towards gene replacement therapy for ovarian cancer a Phase I/II trial to assess the pharmacokinetics and toxicity of intraperitoneal vector therapy was conducted. Clinical grade retroviral vector was produced under cGMP (current Good Manufacturing Practices) and tested for titer(5×10^7 /ml), sterility, and in vitro efficacy. Following placement of an indwelling port-a-cath in patients, a dose escalation study was performed of four daily intraperitoneal infusions spanning doses from 3 mls to 300 mls at half-log intervals (23 cycles in 12 patients). Pharmacokinetics was assessed by PCR and southern blots detecting vector DNA and toxicity was evaluated by clinical exam and fluid analysis.

Results. Three of 12 patients developed an acute sterile peritonitis which spontaneously resolved within 48 hours. This presentation resembled that noted in immunocompetent mice given vector during oyster glycogen induced chronic peritonitis. Plasma antibodies to the retroviral envelope protein were detected in only 1 patient three months after initial treatment, but not in others despite repeat dosing for an interval of up to 4 months. PCR analysis of patient post-treatment peritoneal fluids revealed stable, transduction capable vector 24 hours after infusion. The presence of stable vector correlated inversely with peritoneal CH50 levels supporting the presumed link between complement activation and retroviral vector stability. Gene transfer was documented by PCR, southern blot, western blot, and immunohistochemistry. Eight patients showed disease stabilization for 4 to 16 weeks and three of these showed an objective response with diminished miliary tumor implants at reoperation (2 patients) and radiographic shrinkage of measurable disease (1 patient).

30

Conclusions. The vector-related complication of peritonitis was observed in

three patients but resolved quickly as in preclinical mouse studies.
Intraperitoneal infusion of retroviral vector produces stable vector,
particularly in a subclass of patients with low peritoneal fluid CH50 levels.

Inhibition

5 Detailed Discussion

Retroviruses are known to be rapidly inactivated by complement
present in human sera. Welsh R.M., et al. *Nature* 257: 612-614, 1975;
Ayesh S.K., et al. *Blood* 85: 3503-3509, 1995; Pensiero M.N., et al. *Human*
10 *Gene Therapy* 7:1095-1101,1996; Rother R.P., et al., *Hum. Gene Therapy*
6: 429-435,1995; but are considerably more stable in human compartments
with lower complement levels, Arteaga, C.L., et al. *Cancer Research*
56:1098 1103,1996, suggesting that the peritoneal cavity may represent a
favored site for retroviral vectors. Herein is reported a Phase I trial
evaluating toxicity and pharmacokinetics in 12 patients with ovarian cancer
15 who were intraperitoneally infused with 108- 1010/day of the BRCA1
expressing retroviral vector, LXS-N-BRCA1.

METHODS

Patient Selection and Eligibility Criteria

Patients with recurrent or persistent metastatic epithelial ovarian
20 cancer previously treated with standard surgery and chemotherapy were
considered for study. Inclusion criteria included measurable tumor in 2
dimensions confined to the peritoneal cavity, age >18 and <75,
Gynecologic Oncology Group (GOG) performance status <2, life
expectancy of greater than 3 months, 4 week interval from previous surgery
25 and/or cancer therapy, adequate hematological (WBC >4000/mm³), hepatic
(bilirubin <2mg/dl, SGOT <2x normal), and renal (creatinine <1.5mg/dl)
functions.

Vector Production and Testing

Retroviral vector was manufactured under GMP (Good Manufacturing
30 Practices) conditions employing a CellCube (Corning-Costar, Elmira, NY)
apparatus perfused with Aim V media under continuous monitoring of pH
and O₂. Once the lactate production or glucose consumption are consistent

and appropriate, supernatant is collected as long as the lactate and glucose levels assure optimal vector production. The titer of the vector preparations was determined by quantifying the number of particles present which conferred G418 resistance to transduced MCF-7 cells, employing appropriate dilutions. Vector from this production lot tested negative for bacterial, mycoplasma, and viral contamination and was endotoxin negative. Replication-competent retroviruses could not be detected using PG4 indicator cells following amplification on Mus Dundi.

Study Design

Patients underwent initial placement of a peritoneal portacath for access to the peritoneal cavity followed by admission to the Clinical Research Center at Vanderbilt University Medical Center for treatment. Patients were treated for 4 consecutive days with intraperitoneal LXS-
BRCA1 gene therapy. Five dose levels were studied: 10⁸, 3.3x10⁸, 10⁹, 3.3x10⁹, and 10¹⁰ viral particles. Upon retreatment, patients were escalated to the next highest dose level activated by new patient accrual. Daily blood and peritoneal samples were collected to evaluate for viral uptake by cells, presence of apoptosis, expression of BRCA1 gene, and peritoneal fluid CH50 levels. At 4 week intervals patients were evaluated for response to therapy; if tumor measurements were stable or decreased, retreatment was allowed. Patients who demonstrated tumor progression were evaluated at monthly intervals until death at which time autopsy was requested to evaluate for the systemic presence of retroviral particles and sites of tumor progression.

Detection of vector stability and expression:

DNA was prepared from cell samples by hypotonic lysis followed by digestion with pronase and SDS, followed by pheno/chloroform extraction and ethanol precipitation. DNA was prepared from tissue or tumor samples by freezing samples at -70°C and then finely mincing cold samples with a blade, prior to treatment with proteinase K as described above. RNA was purified from both cells and tumors by lysis in guanidinium thiocyanate by our prior cited methods.

PCR primers specific for the neo sequences within the LXS-
BRCA1

vector were employed for determination of vector presence and stability within patient samples. The primers were 5' CCGGCCGCTTGGGTGGAGA 3' and 5'CAGGTAGCCGGATCAAGCGTATGC 3' and were amplified at the following conditions: initial denaturation at 95°C for 2 minutes; followed by 20 cycles of 1 minute at 94°C, 1 minute at 65°C, and 30 seconds at 72°C. RT-PCR was performed by published methods using the following basic method: RNA samples was reverse transcribed for 1 hour at 37°C using 2 ug of total RNA, 1 ug random hexamers (Boehringer Mannheim), 1X first strand buffer(Gibco BRL), 0.01 M DTT, 0.5 mM each dATP, dCTP, dGTP, and dTTP and 200 U Superscript II RNaseH-reverse transcriptase (Gibco BRL). The RNA:DNA duplexes were used as templates for 20 cycle PCR reactions using the following conditions: denaturation 94°C, 20 seconds; annealing 52°C 45 seconds; elongation 75° C.90 seconds. The following primers were used for RT-PCR studies: LXS-
BRCA1 primers designed to span the LXS LTR and BRCA1 sequences:
5' CCCTCCCTGGGTCAAGCCCTTTGTA 3' and
5'TTCAACGCGAAGAGCAGATAAATCCAT 3'; and control primers for GADPH with sequences: 5' CGCCAGCCGAGCCACATC 3' and 5' AGCCCCAGCCTTCTCCAT 3'.

Southern blotting of Ava I digested DNA was performed with a human BRCA1 probe which was directed exon 24, producing a different sized fragment from vector vis-a-vis normal genomic DNA. Percent transduction was calculated by quantitating hybridization with the phosphoimager and then comparing hybridization of the presumed haploid vector lower band to that of the diploid globin upper band (percent transduction = 2 X vector signal/globin signal).

RESULTS

Twelve patients with recurrent or persistent epithelial ovarian cancer were treated with between 1 and 3 cycles of intraperitoneal vector. These patients included individuals with and without a family history of ovarian or breast cancer representing patients with potentially inherited as well as sporadic ovarian cancer. The clinical features of individual patients are

presented in Table IV.

Toxicity of Intraperitoneal LXS-N-BRCA1 Infusion:

5 An animal model to predict toxicity of LXS-N-BRCA1 in ovarian cancer patients was developed employing prior intraperitoneal oyster glycogen injection in an attempt to mimic peritoneal inflammation often found in malignant effusions. These studies demonstrated that intraperitoneal injection of the LXS-N-BRCA1 vector itself produced a mild peritonitis and focal hepatocellular degeneration in Balb C mice which was dose dependent. However, intraperitoneal administration of LXS-N-BRCA1
10 into oyster glycogen primed animals produced a severe acute peritonitis which killed 2/15 animals in the high dose group. Surviving animals showed rapid resolution of peritonitis over 48 hours with no residual inflammation at 2 weeks. This peritonitis appears to be unique for LXS-N-BRCA1 retroviral vector since a different retroviral vector XM6:antifos, Arteaga, C.L., et al.
15 *Cancer Research* 56:1098-1103, 1996, administered intraperitoneally at similar titer did not produce peritonitis or death in Balb C mice.

Because preclinical toxicity studies produced peritonitis in immunocompetent mice, the patients were carefully evaluated for clinical and laboratory signs of acute peritonitis. Three of the fifteen patients (patients 3,
20 5 and 9) developed peritonitis which resolved within 24 hours after treatment was stopped. Patient 3 was retreated with a lower dose of vector and showed no recurrence of peritonitis, even after dose escalation two further levels. In retrospect, patient 5 was an obese patient with a loculated peritoneal space and may have received a larger than anticipated local dose.
25 Catheter placement is clearly an important consideration in intraperitoneal therapy since delivery of an agent into a confined space likely decreases efficacy and increases risk of local toxicity. Other toxicities in the trial included fever in 4 patients and nausea in 2 patients from the abdominal distension produced by the intraperitoneal infusion of vector.

30 Pharmacokinetics of Intraperitoneal Vector Therapy

Recombinant DNA methods such as southern blotting and polymerase chain reaction (PCR) permit sensitive and specific detection of retroviral

vectors in patient fluids and biopsied tissues. Multiple PCR analyses on plasma samples showed no detectable vector distribution to the systemic circulation, even in patients treated at the highest dose. Twenty-four hours after each infusion (just prior to the next dose) we sampled peritoneal fluid to assess stability and uptake of the retroviral vector. PCR detection of stable vector in peritoneal fluid samples from treated patients was shown. Fluid samples were centrifuged in order to obtain distinct samples for stable vector in peritoneal supernatant (PCR fluid) as well as vector which had entered cells within peritoneal fluid (PCR pellet). Because PCR fluid determinations were performed on 5 ul of peritoneal supernatant and PCR pellet determinations were performed on cells from as much as 10 mls of peritoneal fluid, the PCR pellet assay has greater sensitivity (can detect smaller quantities of vector). Because PCR analysis can detect either transduction-capable vector or degraded vector DNA, 200 ul of patient peritoneal fluid was assayed for the capacity to transduce MCF-7 target cells.

Results of this study demonstrate that LXS_N-BRCA1 vector is still transduction-capable 24 hours after infusion in some samples. Table V shows results from three different PCR-based methods for assessing vector stability and gene transfer. The results of these assays were quite consistent despite the fact that each measured something slightly different. It was consistently observed that vector assays were much more likely to be positive during the later days of treatment than during the early days of treatment (See Table V).

Because complement is known to inactivate retroviruses and since vector stability did not correlate cleanly with vector dose, complement levels in patient fluid samples were assayed and were compared with the PCR-based stability results. These results show an apparent relationship between complement level and vector stability. Although there is no obvious correlation between initial CH50 or mean CH50 and vector stability in patients, samples with low CH50s are more likely to be positive than are those samples with higher CH50s (Table V).

Antibodies could also effect vector stability so patient sera and peritoneal fluid were tested for the development of antibodies to the

amphotropic envelope. The majority of patients never developed detectable antibodies, but one patient (patient 3) developed antibodies after 3 months in both sera and peritoneal fluid. Antibodies did not eliminate vector from the peritoneal fluid since positive PCR samples occurred after the development of antibodies by this patient. Table V shows vector stability, complement levels, and plasma antibody results in treated patients.

Gene transfer into patient cells and tissues was analyzed by PCR, southern blot, and RT-PCR. DNA was purified from peritoneal fluid cells analyzed by PCR which demonstrated transfer of vector into cells within the malignant effusion. Because sampling cells within peritoneal fluid would not necessarily predict gene transfer into malignant or normal tissues, biopsies were obtained from patients who had laparotomies following intraperitoneal treatment. These results showed more efficient integration of vector into the tumor surface than into inner regions of the tumor, and show greater transduction into tumor tissue than into normal tissues. Estimation of transduction rate indicates that 5-10% of cells were transduced with vector in samples which exhibited the strongest signals. In order to assess expression of the retroviral vector, PCR primers were designed which would only detect transcripts which initiated in the retroviral vector and then employed RT-PCR as a semi-quantitative measure of BRCA1 vector expression. These results showed comparatively strong expression of the vector in samples from patients with significant vector transduction who had been recently treated with vector.

Disease stabilization was noted in 8 patients with an objective response defined as a decrease in number of peritoneal miliary implants in 2 patients undergoing reoperation for complications related to their cancer and 1 patient demonstrating decrease in measurable tumor dimensions radiographically. Histologic examination of samples from the 2 patients showing a decrease in miliary implants showed tumor necrosis and granulation tissue in tumors within the peritoneum, but these effects were absent in tumor at distant sites obtained at the autopsy for patient 10. These results are compatible with a localized effect of LXSN-BRCA1 which cannot

affect tumor growth by a systemic mechanism.

5 This Phase I study of LXS_N-BRCA1 demonstrated that the retroviral vector was stable in peritoneal fluid and transferred the gene into cancer cells which expressed the vector. Peritonitis was observed in three patients but resolved rapidly and was analogous to the peritonitis observed in mouse preclinical models. Retreatment does not increase toxicity and does not effect vector stability. Vector inactivation by complement is present in vivo, but antibody development occurs rarely and does not eliminate the vector.

10 Gene therapy has been heralded as disease-specific therapy with few side effects, but the identification of toxicities specifically associated with gene therapy should not be surprising. The LXS_N-BRCA1 peritonitis observed in mice and in certain patients is rapidly reversible and appears to resolve without sequelae. The peritonitis is not clearly dose-related in patients to date although administration into larger numbers of patients may demonstrate a relationship with dose. The peritonitis does not reproducibly occur in a given patient since at least one patient with peritonitis was retreated without recurrence.

20 This protocol employed repeat administration in a number of patients for periods ranging 2-4 months. Antibody formation was rarely observed and neither antibody production nor repeat administration appeared to decrease vector stability. These data suggest that patients may be given repeat doses of retroviral vectors without development of tolerance or enhanced toxicity. Repeat administration increases the cumulative dose of retroviral vector which can be administered and ultimately increases the multiplicity of infection. The highest dose level employed 4 daily injections totalling 6×10^{10} vector particles each month. Since intraperitoneal tumor burdens may be as high as 10^{11} tumor cells (10^{12} cells is known to cause host death) in different patients, it may be very important to increase the dose since these studies appear to be employing a minimal multiplicity of infection.

30 Decreased levels of complement in peritoneal effusions appear to explain the relative stability of vector in this site, so it is important to

consider that vector stability may be a function of both vector dose and complement activity within the patient's peritoneal cavity. One can envision a number of approaches to enhancing the stability of retroviral vectors including complement blockade with lectins or engineering vector envelopes resistant to complement, Rother R.P., et al., *J. Exp. Med.* **182**: 1345-1355, 1995; Rollins S.A., et al. *Hum. Gene Ther.* **7**:619-626, 1996. These types of approaches could expand the population of patients with stable intraperitoneal vector and might permit stable vector in other sites as well.

Retroviral vector therapy with LXS_N-BRCA1 is a rational therapeutic approach which attempts to attack a tumor with the appropriate tumor suppressor gene. Intraperitoneal therapy of ovarian cancer with LXS_N-BRCA1 has a number of clinical advantages, including: 1) natural history of ovarian cancer confinement to peritoneal cavity; 2) known active tumor suppressor gene; 2) peritoneal site permits high dose delivery and vector stability; 4) regional therapy for ovarian cancer is a well-described therapeutic modality 5) current treatment strategies have offered little improvement in survival from ovarian cancer. This human gene therapy model system should allow testing of improved vectors and approaches which may ultimately applied to a myriad of diseases.

TABLE IV

Patient	Age	Stage	Histology	Fam Hx.	Prior Chemo Cycles	Dose Levels	Cycles	Toxicity	Response
1	49	IV	Papillary serous Grade 3	Negative	4	Level 1	1	None	Progression
2	29	IIIB	Papillary serous	Negative	multiple	Level 1	2	None	Stabilization
3	49	IV	Papillary serous	Negative	2	Levels 1-4	4	Fever (101.3) peritonitis	Response
4	42	IV	Papillary serous Grade 3	Breast Cancer	multiple	Level 2	2	None	Stabilization
5	49	IC	Clear Cell carcinoma Grade 3	Breast Cancer (mother)	3	Level 2	1	Fever peritonitis	Progression
6	62	IIIC	Papillary serous Grade 3	Breast Cancer (2 relatives)	1	Level 2-3	3	Congestive Heart Failure	Stabilization
7	52	IIIC	Papillary serous Grade 2-3	Negative	multiple	Level 3	2	None	Stabilization
8	47	IIIC	Papillary serous	Negative	2	Level 4	2	None	Stabilization
9	47	IIIB	Papillary serous	Ovarian CA (2 relatives) Breast (1)	multiple	Level 4-5	3	Fever, myalgia nausea	Response
10	58	IIIC	Clear cell features Grade 3	Endometrial Cancer	3	Level 5	1	Temperature (100.3)	Response
11	55	IIIC	Papillary serous Grade 3	Negative	3	Level 5	2	Fever (102) nausea	Stabilization
12	70	IIIC	Adenocarcinoma Grade 3	Prior Breast Cancer	3	Level 5	2	Nausea	Progression

TABLE V

Pt #	Dose Level	PCR Plasma	PCR Fluid	PCR Pellet	Vector Transfer	Days 1,2	Days 3,4	Initial CH50	Mean CH50	# of CH50's <10	Envelope Antibody	Percent Transduction.
1	1	0/14	0/14	0/8	0/8	-	-	<10	72	1	-	Neg
2	1	0/14	+1/14	0/14	+1/14	-	+	88	61	4	-	NA
3	1-3	0/18	+1/18	0/22	+3/14	+	+	100	73	6	+	10%
4	2	0/8	0/14	0/2	+1/3	-	+	10	3	2	-	Neg
5	2											NA
6	2-3	0/8	+3/12	+3/12	+1/11	-	+	<10	<10	12	-	5%
7	3	0/10	0/10	+3/10	+3/10	-	+	180	100	1	-	5%
8	4	0/10	0/10	0/8	+1/10	-	+	135	93	0	-	NA
9	4-5	0/4	0/8	+1/4	0/2	-	+					NA
10	5			0/5								10%
11	5			0/8								NA
12	5			0/6								NA

SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- (i) APPLICANT: HOLT, JEFFREY T.
JENSEN, ROY A.
5 PAGE, DAVID L.
KING, MARY-CLAIRE
SZABO, CSILLA I.
JETTON, THOMAS L.
ROBINSON-BENION, CHERYL L.
10 THOMPSON, MARILYN E.
- (ii) TITLE OF INVENTION: CHARACTERIZED BRCA1 AND
BRCA2 PROTEINS AND SCREENING AND
THERAPEUTIC METHODS BASED ON
CHARACTERIZED BRCA1 AND BRCA2 PROTEINS.
- 15 (iii) NUMBER OF SEQUENCES: 7
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(C) CITY: NASHVILLE
20 (D) STATE: TENNESSEE
(E) COUNTRY: USA
(F) ZIP: 37219
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Diskette, 3.50 inch, 800 kB storage
25 (B) COMPUTER: IBM PC/XT/AT compatible
(C) OPERATING SYSTEM: Windows 95
(D) SOFTWARE: Microsoft Word 6.0
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER: 08/603,753
30 (B) FILING DATE: 20 FEB 1996
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: U.S. 08/373,799
(B) FILING DATE: 17 JAN 1995
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- (B) REGISTRATION NUMBER: 39,395
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5 (B) TELEFAX: (615) 242-2221
(C) TELEX:
(2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5712
10 (B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA to mRNA
(iii) HYPOTHETICAL: no
15 (iv) ANTI-SENSE: no
(v) ORIGINAL SOURCE
(A) ORGANISM: Homo sapiens
(C) INDIVIDUAL/ISOLATE:
(D) DEVELOPMENTAL STAGE: adult
20 (F) TISSUE TYPE: female breast
(G) CELL TYPE: ductal carcinoma in situ, invasive breast
cancer and normal breast tissue
(H) CELL LINE: not derived from a cell line
(I) ORGANELLE: no
- 25 (vii) IMMEDIATE SOURCE:
(A) LIBRARY: cDNA library derived from human
(B) CLONE: obtained using published sequence
- (viii) POSITION IN GENOME:
30 (A) CHROMOSOME/SEGMENT: unknown
(B) MAP POSITION: unknown
(C) UNITS: unknown
- (ix) FEATURE:
35 (A) NAME/KEY: BRCA1
(B) LOCATION: GenBank accession no. U14680
(C) IDENTIFICATION METHOD:
microscopically-directed sampling and nuclease

54

protection assay

(D) OTHER INFORMATION: gene encoding BRCA1 protein

(x) PUBLICATION INFORMATION:

5 (A) AUTHORS: Miki, Y., et. al.

(B) TITLE: A strong candidate gene for the breast and ovarian cancer susceptibility gene BRCA1.

(C) JOURNAL: Science

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(F) DATE: 1994

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15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

agctcgctga gaattcctgg accccgcacc aggtgtgtgg gttttcaga taactggggc 60

cctgcgctca ggaggccttc accctctgct ctgggtaag ttcattggaa cagaagaa 119

20

atg gat tta tct gct ctt cgc gtt gaa gaa gta caa aat gtc att aat 167
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 1 5 10 15

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 Ala Met Gln Lys Ile Leu Glu Cys Pro Ile Cys Leu Glu Leu Ile Lys
 20 25 30

30

gaa cct gtc tcc aca aag tgt gac cac ata ttt tgc aaa ttt tgc atg 263
 Glu Pro Val Ser Thr Lys Cys Asp His Ile Phe Cys Lys Phe Cys Met
 35 40 45

35

ctg aaa ctt ctc aac cag aag aaa ggg cct tca cag tgt cct tta tgt 311
 Leu Lys Leu Leu Asn Gln Lys Lys Gly Pro Ser Gln Cys Pro Leu Cys
 50 55 60

40

aag aat gat ata acc aaa agg agc cta caa gaa agt acg aga ttt agt 359
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 65 70 75 80

caa ctt gtt gaa gag cta ttg aaa atc att tgt gct ttt cag ctt gac 407
 Gln Leu Val Glu Glu Leu Leu Lys Ile Ile Cys Ala Phe Gln Leu Asp
 85 90 95

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aca ggt ttg gag tat gca aac agc tat aat ttt gca aaa aag gaa aat 455
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10	ggc tac aga aac cgt gcc aaa aga ctt cta cag agt gaa ccc gaa aat Gly Tyr Arg Asn Arg Ala Lys Arg Leu Leu Gln Ser Glu Pro Glu Asn 130 135 140	551		
15	cct tcc ttg cag gaa acc agt ctc agt gtc caa ctc tct aac ctt gga Pro Ser Leu Gln Glu Thr Ser Leu Ser Val Gln Leu Ser Asn Leu Gly 145 150 155 160	599		
20	act gtg aga act ctg agg aca aag cag cgg ata caa cct caa aag acg Thr Val Arg Thr Leu Arg Thr Lys Gln Arg Ile Gln Pro Gln Lys Thr 165 170 175	647		
25	tct gtc tac att gaa ttg gga tct gat tct tct gaa gat acc gtt aat Ser Val Tyr Ile Glu Leu Gly Ser Asp Ser Ser Glu Asp Thr Val Asn 180 185 190	695		
30	aag gca act tat tgc agt gtg gga gat caa gaa ttg tta caa atc acc Lys Ala Thr Tyr Cys Ser Val Gly Asp Gln Glu Leu Leu Gln Ile Thr 195 200 205	743		
35	cct caa gga acc agg gat gaa atc agt ttg gat tct gca aaa aag gct Pro Gln Gly Thr Arg Asp Glu Ile Ser Leu Asp Ser Ala Lys Lys Ala 210 215 220	791		
40	gct tgt gaa ttt tct gag acg gat gta aca aat act gaa cat cat caa Ala Cys Glu Phe Ser Glu Thr Asp Val Thr Asn Thr Glu His His Gln 225 230 235 240	839		
45	ccc agt aat aat gat ttg aac acc act gag aag cgt gca gct gag agg Pro Ser Asn Asn Asp Leu Asn Thr Thr Glu Lys Arg Ala Ala Glu Arg 245 250 255	887		
50	cat cca gaa aag tat cag ggt agt tct gtt tca aac ttg cat gtg gag His Pro Glu Lys Tyr Gln Gly Ser Ser Val Ser Asn Leu His Val Glu 260 265 270	935		
	cca tgt ggc aca aat act cat gcc agc tca tta cag cat gag aac agc Pro Cys Gly Thr Asn Thr His Ala Ser Ser Leu Gln His Glu Asn Ser 275 280 285	983		
	agt tta tta ctc act aaa gac aga atg aat gta gaa aag gct gaa ttc Ser Leu Leu Leu Thr Lys Asp Arg Met Asn Val Glu Lys Ala Glu Phe 290 295 300	1031		
	tgt aat aaa agc aaa cag cct ggc tta gca agg agc caa cat aac aga Cys Asn Lys Ser Lys Gln Pro Gly Leu Ala Arg Ser Gln His Asn Arg	1079		

56

	305	310	315	320	
5	tgg gct gga agt aag gaa aca tgt aat gat agg cgg act ccc agc aca Trp Ala Gly Ser Lys Glu Thr Cys Asn Asp Arg Arg Thr Pro Ser Thr	325	330	335	1127
10	gaa aaa aag gta gat ctg aat gct gat ccc ctg tgt gag aga aaa gaa Glu Lys Lys Val Asp Leu Asn Ala Asp Pro Leu Cys Glu Arg Lys Glu	340	345	350	1175
15	tgg aat aag cag aaa ctg cca tgc tca gag aat cct aga gat act gaa Trp Asn Lys Gln Lys Leu Pro Cys Ser Glu Asn Pro Arg Asp Thr Glu	355	360	365	1223
20	gat gtt cct tgg ata aca cta aat agc agc att cag aaa gtt aat gag Asp Val Pro Trp Ile Thr Leu Asn Ser Ser Ile Gln Lys Val Asn Glu	370	375	380	1271
25	tgg ttt tcc aga agt gat gaa ctg tta ggt tct gat gac tca cat gat Trp Phe Ser Arg Ser Asp Glu Leu Leu Gly Ser Asp Asp Ser His Asp	385	390	395	1319
30	ggg gag tct gaa tca aat gcc aaa gta gct gat gta ttg gac gtt cta Gly Glu Ser Glu Ser Asn Ala Lys Val Ala Asp Val Leu Asp Val Leu	405	410	415	1367
35	aat gag gta gat gaa tat tct ggt tct tca gag aaa ata gac tta ctg Asn Glu Val Asp Glu Tyr Ser Gly Ser Ser Glu Lys Ile Asp Leu Leu	420	425	430	1415
40	gcc agt gat cct cat gag gct tta ata tgt aaa agt gaa aga gtt cac Ala Ser Asp Pro His Glu Ala Leu Ile Cys Lys Ser Asp Arg Val His	435	440	445	1463
45	tcc aaa tca gta gag agt aat att gaa gac aaa ata ttt ggg aaa acc Ser Lys Ser Val Glu Ser Asp Ile Glu Asp Lys Ile Phe Gly Lys Thr	450	455	460	1511
50	tat cgg aag aag gca agc ctc ccc aac tta agc cat gta act gaa aat Tyr Arg Lys Lys Ala Ser Leu Pro Asn Leu Ser His Val Thr Glu Asn	465	470	475	1559
	cta att ata gga gca ttt gtt act gag cca cag ata ata caa gag cgt Leu Ile Ile Gly Ala Phe Val Ser Glu Pro Gln Ile Ile Gln Glu Arg	485	490	495	1607
	ccc ctc aca aat aaa tta aag cgt aaa agg aga cct aca tca ggc ctt Pro Leu Thr Asn Lys Leu Lys Arg Lys Arg Arg Pro Thr Ser Gly Leu	500	505	510	1655
	cat cct gag gat ttt atc aag aaa gca gat ttg gca gtt caa aag act His Pro Glu Asp Phe Ile Lys Lys Ala Asp Leu Ala Val Gln Lys Thr	515	520	525	1703

5	cct gaa atg ata aat cag gga act aac caa acg gag cag aat ggt caa	1751
	Pro Glu Met Ile Asn Gln Gly Thr Asn Gln Thr Glu Gln Asn Gly Gln	
	530 535 540	
10	gtg atg aat att act aat agt ggt cat gag aat aaa aca aaa ggt gat	1799
	Val Met Asn Ile Thr Asn Ser Gly His Glu Asn Lys Thr Lys Gly Asp	
	545 550 555 560	
15	tct att cag aat gag aaa aat cct aac cca ata gaa tca ctc gaa aaa	1847
	Ser Ile Gln Asn Glu Lys Asn Pro Asn Pro Ile Glu Ser Leu Glu Lys	
	565 570 575	
20	gaa tct gct ttc aaa acg aaa gct gaa cct ata agc agc agt ata agc	1895
	Glu Ser Ala Phe Lys Thr Lys Ala Glu Pro Ile Ser Ser Ser Ile Ser	
	580 585 590	
25	aat atg gaa ctc gaa tta aat atc cac aat tca aaa gca cct aaa aag	1943
	Asn Glu Leu Glu Leu Asn Ile Met His Asn Ser Lys Ala Pro Lys Lys	
	595 600 605	
30	aat agg ctg agg agg aag tct tct acc agg cat att cat gcg ctt gaa	1991
	Asn Arg Leu Arg Arg Lys Ser Ser Thr Arg His Ile His Ala Leu Glu	
	610 615 620	
35	cta gta gtc agt aga aat cta agc cca cct aat tgt act gaa ttg caa	2039
	Leu Val Val Ser Arg Asn Leu Ser Pro Pro Asn Cys Thr Glu Leu Gln	
	625 630 635 640	
40	att gat agt tgt tct agc agt gaa gag ata aag aaa aag tac aac	2087
	Ile Asp Ser Cys Ser Ser Ser Glu Glu Ile Lys Lys Lys Lys Tyr Asn	
	645 650 655	
45	caa atg cca gtc agg cac agc aga aac cta caa ctc atg gaa ggt aaa	2135
	Gln Met Pro Val Arg His Ser Arg Asn Leu Gln Leu Met Glu Gly Lys	
	660 665 670	
50	gaa cct gca act gga gcc aag aag agt aac aag cca aat gaa cag aca	2183
	Glu Pro Ala Thr Gly Ala Lys Lys Ser Asn Lys Pro Asn Glu Gln Thr	
	675 680 685	
55	agt aaa aga cat gac agc gat act ttc cca gag ctg aag tta aca aat	2231
	Ser Lys Arg His Asp Ser Asp Thr Phe Pro Glu Leu Lys Leu Thr Asn	
	690 695 700	
60	gca cct ggt tct ttt act aag tgt tca aat acc agt gaa ctt aaa gaa	2279
	Ala Pro Gly Ser Phe Thr Lys Cys Ser Asn Thr Ser Glu Leu Lys Glu	
	705 710 715 720	
65	ttt gtc aat cct agc ctt cca aga gaa gaa aaa gaa gag aaa cta gaa	2327
	Phe Val Asn Pro Ser Leu Pro Arg Glu Glu Lys Glu Glu Lys Leu Glu	
	725 730 735	

58

	aca gtt aaa gtg tct aat aat gct gaa gac ccc aaa gat ctc atg tta	2375
	Thr Val Lys Val Ser Asn Asn Ala Glu Asp Pro Lys Asp Leu Met Leu	
	740 745 750	
5	agt gga gaa agg gtt ttg caa act gaa aga tct gta gag agt agc agt	2423
	Ser Gly Glu Arg Val Leu Gln Thr Glu Arg Ser Val Glu Ser Ser Ser	
	755 760 765	
10	att tca ttg gta cct ggt act gat tat ggc act cag gaa agt atc tcg	2471
	Ile Ser Leu Val Pro Gly Thr Asp Tyr Gly Thr Gln Glu Ser Ile Ser	
	770 775 780	
15	tta ctg gaa gtt agc act cta ggg aag gca aaa aca gaa cca aat aaa	2519
	Leu Leu Glu Val Ser Thr Leu Gly Lys Ala Lys Thr Glu Pro Asn Lys	
	785 790 795 800	
20	tgt gtg agt cag tgt gca gca ttt gaa aac ccc aag gga cta att cat	2567
	Cys Val Ser Gln Cys Ala Ala Phe Glu Asn Pro Lys Gly Leu Ile His	
	805 810 815	
	ggg tgt tcc aaa gat aat aga aat gac aca gaa ggc ttt aag tat cca	2615
	Gly Cys Ser Lys Asp Asn Arg Asn Asp Thr Glu Gly Phe Lys Tyr Pro	
	820 825 830	
25	ttg gga cat gaa gtt aac cac agt cgg gaa aca agc ata gaa atg gaa	2663
	Leu Gly His Glu Val Asn His Ser Arg Glu Thr Ser Ile Glu Met Glu	
	835 840 845	
30	gaa agt gaa ctt gat gct cag tat ttg cag aat aca ttc aag gtt tca	2711
	Glu Ser Glu Leu Asp Ala Gln Tyr Leu Gln Asn Thr Phe Lys Val Ser	
	850 855 860	
35	aag cgc cag tca ttt gct ccg ttt tca aat cca gga aat gca gaa gag	2759
	Lys Arg Gln Ser Phe Ala Pro Phe Ser Asn Pro Gly Asn Ala Glu Glu	
	865 870 875 880	
40	gaa tgt gca aca ttc tct gcc cac tct ggg tcc tta aag aaa caa agt	2807
	Glu Cys Ala Thr Phe Ser Ala His Ser Gly Ser Leu Lys Lys Gln Ser	
	885 890 895	
	cca aaa gtc act ttt gaa tgt gaa caa aag gaa gaa aat caa gga aag	2855
	Pro Lys Val Thr Phe Glu Cys Glu Gln Lys Glu Glu Asn Gln Gly Lys	
	900 905 910	
45	aat gag tct aat atc aag cct gta cag aca gtt aat atc act gca ggc	2903
	Asn Glu Ser Asn Ile Lys Pro Val Gln Thr Val Asn Ile Thr Ala Gly	
	915 920 925	
50	ttt cct gtg gtt ggt cag aaa gat aag cca gtt gat aat gcc aaa tgt	2951
	Phe Pro Val Val Gly Gln Lys Asp Lys Pro Val Asp Asn Ala Lys Cys	
	930 935 940	
	agt atc aaa gga ggc tct agg ttt tgt cta tca tct cag ttc aga ggc	2999

59

	Ser Ile Lys Gly Gly Ser Arg Phe Cys Leu Ser Ser Gln Phe Arg Gly	
	945 950 955 960	
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	Asn Glu Thr Gly Leu Ile Thr Pro Asn Lys His Gly Leu Leu Gln Asn	
	965 970 975	
10	cca tat cgt ata cca cca ctt ttt ccc atc aag tca ttt gtt aaa act	3095
	Pro Tyr Arg Ile Pro Pro Leu Phe Pro Ile Lys Ser Phe Val Lys Thr	
	980 985 990	
15	aaa tgt aag aaa aat ctg cta gag gaa aac ttt gag gaa cat tca atg	3143
	Lys Cys Lys Lys Asn Leu Leu Glu Glu Asn Phe Glu Glu His Ser Met	
	995 1000 1005	
20	tca cct gaa aga gaa atg gga aat gag aac att cca agt aca gtg agc	3191
	Ser Pro Glu Arg Glu Met Gly Asn Glu Asn Ile Pro Ser Thr Val Ser	
	1010 1015 1020	
25	aca att agc cgt aat aac att aga gaa aat gtt ttt aaa gaa gcc agc	3239
	Thr Ile Ser Arg Asn Asn Ile Arg Glu Asn Val Phe Lys Glu Ala Ser	
	1025 1030 1035 1040	
30	tca agc aat att aat gaa gta ggt tcc agt act aat gaa gtg ggc tcc	3287
	Ser Ser Asn Ile Asn Glu Val Gly Ser Ser Thr Asn Glu Val Gly Ser	
	1045 1050 1055	
35	agt att aat gaa ata ggt tcc agt gat gaa aac att caa gca gaa cta	3335
	Ser Ile Asn Glu Ile Gly Ser Ser Asp Glu Asn Ile Gln Ala Glu Leu	
	1060 1065 1070	
40	ggc aga aac aga ggg cca aaa ttg aat gct atg ctt aga tta ggg gtt	3383
	Gly Arg Asn Arg Gly Pro Lys Leu Asn Ala Met Leu Arg Leu Gly Val	
	1075 1080 1085	
45	ttg caa cct gag gtc tat aaa caa agt ctt cct gga agt aat tgt aag	3431
	Leu Gln Pro Glu Val Tyr Lys Gln Ser Leu Pro Gly Ser Asn Cys Lys	
	1090 1095 1100	
50	cat cct gaa ata aaa aag caa gaa tat gaa gaa gta gtt cag act gtt	3479
	His Pro Glu Ile Lys Lys Gln Glu Tyr Glu Glu Val Val Gln Thr Val	
	1105 1110 1115 1120	
55	aat aca gat ttc tct cca tat ctg att tca gat aac tta gaa cag cct	3527
	Asn Thr Asp Phe Ser Pro Tyr Leu Ile Ser Asp Asn Leu Glu Gln Pro	
	1125 1130 1135	
60	atg gga agt agt cat gca tct cag gtt tgt tct gag aca cct gat gac	3575
	Met Gly Ser Ser His Ala Ser Gln Val Cys Ser Glu Thr Pro Asp Asp	
	1140 1145 1150	
65	ctg tta gat gat ggt gaa ata aag gaa gat act agt ttt gct gaa aat	3623
	Leu Leu Asp Asp Gly Glu Ile Lys Glu Asp Thr Ser Phe Ala Glu Asn	

60

	1155	1160	1165	
5	gac att aag gaa agt tct gct gtt ttt agc aaa agc gtc cag aaa gga Asp Ile Lys Glu Ser Ser Ala Val Phe Ser Lys Ser Val Gln Lys Gly 1170 1175 1180	3671		
10	gag ctt agc agg agt cct agc cct ttc acc cat aca cat ttg gct cag Glu Leu Ser Arg Ser Pro Ser Pro Phe Thr His Thr His Leu Ala Gln 1185 1190 1195 1200	3719		
15	ggc tac cga aga ggg gcc aag aaa tta gag tcc tca gaa gag aac tta Gly Tyr Arg Arg Gly Ala Lys Lys Leu Glu Ser Ser Glu Glu Asn Leu 1205 1210 1215	3767		
20	tct agt gag gat gaa gag ctt ccc tgc ttc caa cac ttg tta ttt ggt Ser Ser Glu Asp Glu Glu Leu Pro Cys Phe Gln His Leu Leu Phe Gly 1220 1225 1230	3815		
25	aaa gta aac aat ata cct tct cag tct act agg cat agc acc gtt gct Lys Val Asn Asn Ile Pro Ser Gln Ser Thr Arg His Ser Thr Val Ala 1235 1240 1245	3863		
30	acc gag tgt ctg tct aag aac aca gag gag aat tta tta tca ttg aag Thr Glu Cys Leu Ser Lys Asn Thr Glu Glu Asn Leu Leu Ser Leu Lys 1250 1255 1260	3911		
35	aat agc tta aat gac tgc agt aac cag gta ata ttg gca aag gca tct Asn Ser Leu Asn Asp Cys Ser Asn Gln Val Ile Leu Ala Lys Ala Ser 1265 1270 1275 1280	3959		
40	cag gaa cat cac ctt agt gag gaa aca aaa tgt tct gct agc ttg ttt Gln Glu His His Leu Ser Glu Glu Thr Lys Cys Ser Ala Ser Leu Phe 1285 1290 1295	4007		
45	tct tca cag tgc agt gaa ttg gaa gac ttg act gca aat aca aac acc Ser Ser Gln Cys Ser Glu Leu Glu Asp Leu Thr Ala Asn Thr Asn Thr 1300 1305 1310	4055		
50	cag gat cct ttc ttg att ggt tct tcc aaa caa atg agg cat cag tct Gln Asp Pro Phe Leu Ile Gly Ser Ser Lys Gln Met Arg His Gln Ser 1315 1320 1325	4103		
	gaa agc cag gaa gtt ggt ctg agt gac aag gaa ttg gtt tca gat gat Glu Ser Gln Gly Val Gly Leu Ser Asp Lys Glu Leu Val Ser Asp Asp 1330 1335 1340	4151		
	gaa gaa aga gga acg ggc ttg gaa gaa aat aat caa gaa gag caa agc Glu Glu Arg Gly Thr Gly Leu Glu Glu Asn Asn Gln Glu Glu Gln Ser 1345 1350 1355 1360	4199		
	atg gat tca aac tta ggt gaa gca gca tct ggg tgt gag agt gaa aca Met Asp Ser Asn Leu Gly Glu Ala Ala Ser Gly Cys Glu Ser Glu Thr 1365 1370 1375	4247		

	agc gtc tct gaa gac tgc tca ggg cta tcc tct cag agt gac att tta	4295
	Ser Val Ser Glu Asp Cys Ser Gly Leu Ser Ser Gln Ser Asp Ile Leu	
	1380 1385 1390	
5	acc act cag cag agg gat acc atg caa cat aac ctg ata aag ctc cag	4343
	Thr Thr Gln Gln Arg Asp Thr Met Gln His Asn Leu Ile Lys Leu Gln	
	1395 1400 1405	
10	cag gaa atg gct gaa cta gaa gct gtg tta gaa cag cat ggg agc cag	4391
	Gln Glu Met Ala Glu Leu Glu Ala Val Leu Glu Gln His Gly Ser Gln	
	1410 1415 1420	
15	cct tct aac agc tac cct tcc atc ata agt gac tct tct gcc ctt gag	4439
	Pro Ser Asn Ser Tyr Pro Ser Ile Ile Ser Asp Ser Ser Ala Leu Glu	
	1425 1430 1435 1440	
20	gac ctg cga aat cca gaa caa agc aca tca gaa aaa gca gta tta act	4487
	Asp Leu Arg Asn Pro Glu Gln Ser Thr Ser Glu Lys Val Leu Gln Thr	
	1445 1450 1455	
25	tca cag aaa agt agt gaa tac cct ata agc cag aat cca gaa ggc ctt	4535
	Ser Gln Lys Ser Ser Glu Tyr Pro Ile Ser Gln Asn Pro Glu Gly Xaa	
	1460 1465 1470	
30	tct gct gac aag ttt gag gtg tct gca gat agt tct acc agt aaa aat	4583
	Ser Ala Asp Lys Phe Glu Val Ser Ala Asp Ser Ser Thr Ser Lys Asn	
	1475 1480 1485	
35	aaa gaa cca gga gtg gaa agg tca tcc cct tct aaa tgc cca tca tta	4631
	Lys Glu Pro Gly Val Glu Arg Ser Ser Pro Ser Lys Cys Pro Ser Leu	
	1490 1495 1500	
40	gat gat agg tgg tac atg cac agt tgc tct ggg agt ctt cag aat aga	4679
	Asp Asp Arg Trp Tyr Met His Ser Cys Ser Gly Ser Leu Gln Asn Arg	
	1505 1510 1515 1520	
45	aac tac cca tct caa gag gag ctc att aag gtt gtt gat gtg gag gag	4727
	Asn Tyr Pro Pro Gln Glu Glu Leu Ile Lys Val Val Asp Val Glu Glu	
	1525 1530 1535	
50	caa cag ctg gaa gag tct ggg cca cac gat ttg acg gaa aca tct tac	4775
	Gln Gln Leu Glu Glu Ser Gly Pro His Asp Leu Thr Glu Thr Ser Tyr	
	1540 1545 1550	
55	ttg cca agg caa gat cta gag gga acc cct tac ctg gaa tct gga atc	4823
	Leu Pro Arg Gln Asp Leu Glu Gly Thr Pro Tyr Leu Glu Ser Gly Ile	
	1555 1560 1565	
60	agc ctc ttc tct gat gac cct gaa tct gat cct tct gaa gac aga gcc	4871
	Ser Leu Phe Ser Asp Asp Pro Glu Ser Asp Pro Ser Glu Asp Arg Ala	
	1570 1575 1580	

	cca gag tca gct cgt gtt ggc aac ata cca tct tca acc tct gca ttg	4919
	Pro Glu Ser Ala Arg Val Gly Asn Ile Pro Ser Ser Thr Ser Ala Leu	
	1585 1590 1595 1600	
5	aaa gtt ccc caa ttg aaa gtt gca gaa tct gcc cag agt cca gct gct	4967
	Lys Val Pro Gln Leu Lys Val Ala Glu Ser Ala Gln Ser Pro Ala Ala	
	1605 1610 1615	
10	gct cat act act gat act gct ggg tat aat gca atg gaa gaa agt gtg	5015
	Ala His Thr Thr Asp Thr Ala Gly Tyr Asn Ala Met Glu Glu Ser Val	
	1620 1625 1630	
15	agc agg gag aag cca gaa ttg aca gct tca aca gaa agg gtc aac aaa	5063
	Ser Arg Glu Lys Pro Glu Leu Thr Ala Ser Thr Glu Arg Val Asn Lys	
	1635 1640 1645	
20	aga atg tcc atg gtg gtg tct ggc ctg acc cca gaa gaa ttt atg ctc	5111
	Arg Met Ser Met Val Val Ser Gly Leu Thr Pro Glu Glu Phe Met Leu	
	1650 1655 1660	
25	gtg tac aag ttt gcc aga aaa cac cac atc act tta act aat cta att	5159
	Val Tyr Lys Phe Ala Arg Lys His His Ile Thr Leu Thr Asn Leu Ile	
	1665 1670 1675 1680	
30	act gaa gag act act cat gtt gtt atg aaa aca gat gct gag ttt gtg	5207
	Thr Glu Glu Thr Thr His Val Val Met Lys Thr Asp Ala Glu Phe Val	
	1685 1690 1695	
35	tgt gaa cgg aca ctg aaa tat ttt cta gga att gcg gga gga aaa tgg	5255
	Cys Glu Arg Thr Leu Lys Tyr Phe Leu Gly Ile Ala Gly Gly Lys Trp	
	1700 1705 1710	
40	gta gtt agc tat ttc tgg gtg acc cag tct att aaa gaa aga aaa atg	5303
	Val Val Ser Tyr Phe Trp Val Thr Gln Ser Ile Lys Glu Arg Lys Met	
	1715 1720 1725	
45	ctg aat gag cat gat ttt gaa gtc aga gga gat gtg gtc aat gga aga	5351
	Leu Asn Glu His Asp Phe Glu Val Arg Gly Asp Val Val Asn Gly Arg	
	1730 1735 1740	
50	aac cac caa ggt cca aag cga gca aga gaa tcc cag gac aga aag atc	5399
	Asn His Gln Gly Pro Lys Arg Ala Arg Glu Ser Gln Asp Arg Lys Ile	
	1745 1750 1755 1760	
55	ttc agg ggg cta gaa atc tgt tgc tat ggg ccc ttc acc aac atg ccc	5447
	Phe Arg Gly Leu Glu Ile Cys Cys Tyr Gly Pro Phe Thr Asn Met Pro	
	1765 1770 1775	
60	aca gat caa ctg gaa tgg atg gta cag ctg tgt ggt gct tct gtg gtg	5495
	Thr Asp Gln Leu Glu Trp Met Val Gln Leu Cys Gly Ala Ser Val Val	
	1780 1785 1790	
	aag gag ctt tca tca ttc acc ctt ggc aca ggt gtc cac cca att gtg	5543

63

Lys Glu Leu Ser Ser Phe Thr Leu Gly Thr Gly Val His Pro Ile Val
 1795 1800 1805
 gtt gtg cag cca gat gcc tgg aca gag gac aat ggc ttc cat gca att 5591
 5 Val Val Gln Pro Asp Ala Trp Thr Glu Asp Asn Gly Phe His Ala Ile
 1810 1815 1820
 ggg cag atg tgt gag gca cct gtg gtg acc cga gag tgg gtg ttg gac 5639
 10 Gly Gln Met Cys Glu Ala Pro Val Val Thr Arg Glu Trp Val Leu Asp
 1825 1830 1835 1840
 agt gta gca ctc tac cag tgc cag gag ctg gac acc tac ctg ata ccc 5687
 15 Ser Val Ala Leu Tyr Gln Cys Gln Glu Leu Asp Thr Tyr Leu Ile Pro
 1845 1850 1855
 cag atc ccc cac agc cac tac tgat 5712
 20 Gln Ile Pro His Ser His Tyr
 1860

20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1863
 (B) TYPE: amino acid
 25 (C) STRANDEDNESS: single
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

30 (v) ORIGINAL SOURCE

- (A) ORGANISM: Homo sapiens sapiens
 (C) INDIVIDUAL/ISOLATE:
 (D) DEVELOPMENTAL STAGE: adult
 (F) TISSUE TYPE: female breast
 35 (G) CELL TYPE: normal breast tissue
 (H) CELL LINE: not derived from a cell line
 (I) ORGANELLE: no

(ix) FEATURE:

- (A) NAME/KEY: BRCA1 protein
 40 (B) LOCATION: 1 to 1863
 (C) IDENTIFICATION METHOD: observation of mRNA
 and antisense inhibition of BRCA1 gene

(D) OTHER INFORMATION: BRCA1 protein has a negative regulatory effect on growth of human mammary cells.

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Miki, Y., et. al.

5 (B) TITLE: A strong candidate gene for the breast and ovarian cancer susceptibility gene BRCA1.

(C) JOURNAL: Science

(D) VOLUME: 266

10 (E) PAGES: 66-71

(F) DATE: 1994

(K) RELEVANT RESIDUES IN SEQ ID NO:2:
granin box domain at amino acids 1214-1223

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Asp Leu Ser Ala Leu Arg Val Glu Glu Val Gln Asn Val Ile Asn
1 5 10 15

20 Ala Met Gln Lys Ile Leu Glu Cys Pro Ile Cys Leu Glu Leu Ile Lys
20 25 30

25 Glu Pro Val Ser Thr Lys Cys Asp His Ile Phe Cys Lys Phe Cys Met
35 40 45

Leu Lys Leu Leu Asn Gln Lys Lys Gly Pro Ser Gln Cys Pro Leu Cys
50 55 60

30 Lys Asn Asp Ile Thr Lys Arg Ser Leu Gln Glu Ser Thr Arg Phe Ser
65 70 75 80

Gln Leu Val Glu Glu Leu Leu Lys Ile Ile Cys Ala Phe Gln Leu Asp
85 90 95

35 Thr Gly Leu Glu Tyr Ala Asn Ser Tyr Asn Phe Ala Lys Lys Glu Asn
100 105 110

40 Asn Ser Pro Glu His Leu Lys Asp Glu Val Ser Ile Ile Gln Ser Met
115 120 125

Gly Tyr Arg Asn Arg Ala Lys Arg Leu Leu Gln Ser Glu Pro Glu Asn
130 135 140

45 Pro Ser Leu Gln Glu Thr Ser Leu Ser Val Gln Leu Ser Asn Leu Gly
145 150 155 160

65

	Thr Val Arg Thr Leu Arg Thr Lys Gln Arg Ile Gln Pro Gln Lys Thr	
	165	170 175
5	Ser Val Tyr Ile Glu Leu Gly Ser Asp Ser Ser Glu Asp Thr Val Asn	
	180	185 190
	Lys Ala Thr Tyr Cys Ser Val Gly Asp Gln Glu Leu Leu Gln Ile Thr	
	195	200 205
10	Pro Gln Gly Thr Arg Asp Glu Ile Ser Leu Asp Ser Ala Lys Lys Ala	
	210	215 220
	Ala Cys Glu Phe Ser Glu Thr Asp Val Thr Asn Thr Glu His His Gln	
15	225	230 235 240
	Pro Ser Asn Asn Asp Leu Asn Thr Thr Glu Lys Arg Ala Ala Glu Arg	
	245	250 255
	His Pro Glu Lys Tyr Gln Gly Ser Ser Val Ser Asn Leu His Val Glu	
20	260	265 270
	Pro Cys Gly Thr Asn Thr His Ala Ser Ser Leu Gln His Glu Asn Ser	
	275	280 285
25	Ser Leu Leu Leu Thr Lys Asp Arg Met Asn Val Glu Lys Ala Glu Phe	
	290	295 300
	Cys Asn Lys Ser Lys Gln Pro Gly Leu Ala Arg Ser Gln His Asn Arg	
30	305	310 315 320
	Trp Ala Gly Ser Lys Glu Thr Cys Asn Asp Arg Arg Thr Pro Ser Thr	
	325	330 335
	Glu Lys Lys Val Asp Leu Asn Ala Asp Pro Leu Cys Glu Arg Lys Glu	
35	340	345 350
	Trp Asn Lys Gln Lys Leu Pro Cys Ser Glu Asn Pro Arg Asp Thr Glu	
	355	360 365
40	Asp Val Pro Trp Ile Thr Leu Asn Ser Ser Ile Gln Lys Val Asn Glu	
	370	375 380
	Trp Phe Ser Arg Ser Asp Glu Leu Leu Gly Ser Asp Asp Ser His Asp	
45	385	390 395 400
	Gly Glu Ser Glu Ser Asn Ala Lys Val Ala Asp Val Leu Asp Val Leu	
	405	410 415
	Asn Glu Val Asp Glu Tyr Ser Gly Ser Ser Glu Lys Ile Asp Leu Leu	
50	420	425 430
	Ala Ser Asp Pro His Glu Ala Leu Ile Cys Lys Ser Asp Arg Val His	
	435	440 445

66

Ser Lys Ser Val Glu Ser Asp Ile Glu Asp Lys Ile Phe Gly Lys Thr
 450 455 460

5 Tyr Arg Lys Lys Ala Ser Leu Pro Asn Leu Ser His Val Thr Glu Asn
 465 470 475 480

Leu Ile Ile Gly Ala Phe Val Ser Glu Pro Gln Ile Ile Gln Glu Arg
 485 490 495

10 Pro Leu Thr Asn Lys Leu Lys Arg Lys Arg Arg Pro Thr Ser Gly Leu
 500 505 510

His Pro Glu Asp Phe Ile Lys Lys Ala Asp Leu Ala Val Gln Lys Thr
 15 515 520 525

Pro Glu Met Ile Asn Gln Gly Thr Asn Gln Thr Glu Gln Asn Gly Gln
 530 535 540

20 Val Met Asn Ile Thr Asn Ser Gly His Glu Asn Lys Thr Lys Gly Asp
 545 550 555 560

Ser Ile Gln Asn Glu Lys Asn Pro Asn Pro Ile Glu Ser Leu Glu Lys
 25 565 570 575

Glu Ser Ala Phe Lys Thr Lys Ala Glu Pro Ile Ser Ser Ser Ile Ser
 580 585 590

Asn Glu Leu Glu Leu Asn Ile Met His Asn Ser Lys Ala Pro Lys Lys
 30 595 600 605

Asn Arg Leu Arg Arg Lys Ser Ser Thr Arg His Ile His Ala Leu Glu
 610 615 620

35 Leu Val Val Ser Arg Asn Leu Ser Pro Pro Asn Cys Thr Glu Leu Gln
 625 630 635 640

Ile Asp Ser Cys Ser Ser Ser Glu Glu Ile Lys Lys Lys Lys Tyr Asn
 40 645 650 655

Gln Met Pro Val Arg His Ser Arg Asn Leu Gln Leu Met Glu Gly Lys
 660 665 670

Glu Pro Ala Thr Gly Ala Lys Lys Ser Asn Lys Pro Asn Glu Gln Thr
 45 675 680 685

Ser Lys Arg His Asp Ser Asp Thr Phe Pro Glu Leu Lys Leu Thr Asn
 690 695 700

50 Ala Pro Gly Ser Phe Thr Lys Cys Ser Asn Thr Ser Glu Leu Lys Glu
 705 710 715 720

Phe Val Asn Pro Ser Leu Pro Arg Glu Glu Lys Glu Glu Lys Leu Glu

67

	725	730	735
	Thr Val Lys Val Ser Asn Asn Ala Glu Asp Pro Lys Asp Leu Met Leu		
	740	745	750
5	Ser Gly Glu Arg Val Leu Gln Thr Glu Arg Ser Val Glu Ser Ser Ser		
	755	760	765
	Ile Ser Leu Val Pro Gly Thr Asp Tyr Gly Thr Gln Glu Ser Ile Ser		
10	770	775	780
	Leu Leu Glu Val Ser Thr Leu Gly Lys Ala Lys Thr Glu Pro Asn Lys		
	785	790	800
15	Cys Val Ser Gln Cys Ala Ala Phe Glu Asn Pro Lys Gly Leu Ile His		
	805	810	815
	Gly Cys Ser Lys Asp Asn Arg Asn Asp Thr Glu Gly Phe Lys Tyr Pro		
20	820	825	830
	Leu Gly His Glu Val Asn His Ser Arg Glu Thr Ser Ile Glu Met Glu		
	835	840	845
	Glu Ser Glu Leu Asp Ala Gln Tyr Leu Gln Asn Thr Phe Lys Val Ser		
25	850	855	860
	Lys Arg Gln Ser Phe Ala Pro Phe Ser Asn Pro Gly Asn Ala Glu Glu		
	865	870	875
30	Glu Cys Ala Thr Phe Ser Ala His Ser Gly Ser Leu Lys Lys Gln Ser		
	885	890	895
	Pro Lys Val Thr Phe Glu Cys Glu Gln Lys Glu Glu Asn Gln Gly Lys		
35	900	905	910
	Asn Glu Ser Asn Ile Lys Pro Val Gln Thr Val Asn Ile Thr Ala Gly		
	915	920	925
	Phe Pro Val Val Gly Gln Lys Asp Lys Pro Val Asp Asn Ala Lys Cys		
40	930	935	940
	Ser Ile Lys Gly Gly Ser Arg Phe Cys Leu Ser Ser Gln Phe Arg Gly		
	945	950	955
45	Asn Glu Thr Gly Leu Ile Thr Pro Asn Lys His Gly Leu Leu Gln Asn		
	965	970	975
	Pro Tyr Arg Ile Pro Pro Leu Phe Pro Ile Lys Ser Phe Val Lys Thr		
50	980	985	990
	Lys Cys Lys Lys Asn Leu Leu Glu Glu Asn Phe Glu Glu His Ser Met		
	995	1000	1005

68

	Ser Pro Glu Arg Glu Met Gly Asn Glu Asn Ile Pro Ser Thr Val Ser
	1010 1015 1020
5	Thr Ile Ser Arg Asn Asn Ile Arg Glu Asn Val Phe Lys Glu Ala Ser
	1025 1030 1035 1040
	Ser Ser Asn Ile Asn Glu Val Gly Ser Ser Thr Asn Glu Val Gly Ser
	1045 1050 1055
10	Ser Ile Asn Glu Ile Gly Ser Ser Asp Glu Asn Ile Gln Ala Glu Leu
	1060 1065 1070
	Gly Arg Asn Arg Gly Pro Lys Leu Asn Ala Met Leu Arg Leu Gly Val
15	1075 1080 1085
	Leu Gln Pro Glu Val Tyr Lys Gln Ser Leu Pro Gly Ser Asn Cys Lys
	1090 1095 1100
20	His Pro Glu Ile Lys Lys Gln Glu Tyr Glu Glu Val Val Gln Thr Val
	1105 1110 1115 1120
	Asn Thr Asp Phe Ser Pro Tyr Leu Ile Ser Asp Asn Leu Glu Gln Pro
	1125 1130 1135
25	Met Gly Ser Ser His Ala Ser Gln Val Cys Ser Glu Thr Pro Asp Asp
	1140 1145 1150
	Leu Leu Asp Asp Gly Glu Ile Lys Glu Asp Thr Ser Phe Ala Glu Asn
30	1155 1160 1165
	Asp Ile Lys Glu Ser Ser Ala Val Phe Ser Lys Ser Val Gln Lys Gly
	1170 1175 1180
35	Glu Leu Ser Arg Ser Pro Ser Pro Phe Thr His Thr His Leu Ala Gln
	1185 1190 1195 1200
	Gly Tyr Arg Arg Gly Ala Lys Lys Leu Glu Ser Ser Glu Glu Asn Leu
	1205 1210 1215
40	Ser Ser Glu Asp Glu Glu Leu Pro Cys Phe Gln His Leu Leu Phe Gly
	1220 1225 1230
	Lys Val Asn Asn Ile Pro Ser Gln Ser Thr Arg His Ser Thr Val Ala
45	1235 1240 1245
	Thr Glu Cys Leu Ser Lys Asn Thr Glu Glu Asn Leu Leu Ser Leu Lys
	1250 1255 1260
50	Asn Ser Leu Asn Asp Cys Ser Asn Gln Val Ile Leu Ala Lys Ala Ser
	1265 1270 1275 1280
	Gln Glu His His Leu Ser Glu Glu Thr Lys Cys Ser Ala Ser Leu Phe
	1285 1290 1295

	Ser Ser Gln Cys Ser Glu Leu Glu Asp Leu Thr Ala Asn Thr Asn Thr
	1300 1305 1310
5	Gln Asp Pro Phe Leu Ile Gly Ser Ser Lys Gln Met Arg His Gln Ser
	1315 1320 1325
	Glu Ser Gln Gly Val Gly Leu Ser Asp Lys Glu Leu Val Ser Asp Asp
10	1330 1335 1340
	Glu Glu Arg Gly Thr Gly Leu Glu Glu Asn Asn Gln Glu Glu Gln Ser
	1345 1350 1355 1360
15	Met Asp Ser Asn Leu Gly Glu Ala Ala Ser Gly Cys Glu Ser Glu Thr
	1365 1370 1375
	Ser Val Ser Glu Asp Cys Ser Gly Leu Ser Ser Gln Ser Asp Ile Leu
	1380 1385 1390
20	Thr Thr Gln Gln Arg Asp Thr Met Gln His Asn Leu Ile Lys Leu Gln
	1395 1400 1405
	Gln Glu Met Ala Glu Leu Glu Ala Val Leu Glu Gln His Gly Ser Gln
25	1410 1415 1420
	Pro Ser Asn Ser Tyr Pro Ser Ile Ile Ser Asp Ser Ser Ala Leu Glu
	1425 1430 1435 1440
30	Asp Leu Arg Asn Pro Glu Gln Ser Thr Ser Glu Lys Val Leu Gln Thr
	1445 1450 1455
	Ser Gln Lys Ser Ser Glu Tyr Pro Ile Ser Gln Asn Pro Glu Gly Xaa
	1460 1465 1470
35	Ser Ala Asp Lys Phe Glu Val Ser Ala Asp Ser Ser Thr Ser Lys Asn
	1475 1480 1485
	Lys Glu Pro Gly Val Glu Arg Ser Ser Pro Ser Lys Cys Pro Ser Leu
40	1490 1495 1500
	Asp Asp Arg Trp Tyr Met His Ser Cys Ser Gly Ser Leu Gln Asn Arg
	1505 1510 1515 1520
45	Asn Tyr Pro Pro Gln Glu Glu Leu Ile Lys Val Val Asp Val Glu Glu
	1525 1530 1535
	Gln Gln Leu Glu Glu Ser Gly Pro His Asp Leu Thr Glu Thr Ser Tyr
	1540 1545 1550
50	Leu Pro Arg Gln Asp Leu Glu Gly Thr Pro Tyr Leu Glu Ser Gly Ile
	1555 1560 1565
	Ser Leu Phe Ser Asp Asp Pro Glu Ser Asp Pro Ser Glu Asp Arg Ala

70

	1570	1575	1580
5	Pro Glu Ser Ala Arg Val Gly Asn Ile Pro Ser Ser Thr Ser Ala Leu 1585	1590	1595 1600
	Lys Val Pro Gln Leu Lys Val Ala Glu Ser Ala Gln Ser Pro Ala Ala 1605	1610	1615
10	Ala His Thr Thr Asp Thr Ala Gly Tyr Asn Ala Met Glu Glu Ser Val 1620	1625	1630
	Ser Arg Glu Lys Pro Glu Leu Thr Ala Ser Thr Glu Arg Val Asn Lys 1635	1640	1645
15	Arg Met Ser Met Val Val Ser Gly Leu Thr Pro Glu Glu Phe Met Leu 1650	1655	1660
20	Val Tyr Lys Phe Ala Arg Lys His His Ile Thr Leu Thr Asn Leu Ile 1665	1670	1675 1680
	Thr Glu Glu Thr Thr His Val Val Met Lys Thr Asp Ala Glu Phe Val 1685	1690	1695
25	Cys Glu Arg Thr Leu Lys Tyr Phe Leu Gly Ile Ala Gly Gly Lys Trp 1700	1705	1710
	Val Val Ser Tyr Phe Trp Val Thr Gln Ser Ile Lys Glu Arg Lys Met 1715	1720	1725
30	Leu Asn Glu His Asp Phe Glu Val Arg Gly Asp Val Val Asn Gly Arg 1730	1735	1740
35	Asn His Gln Gly Pro Lys Arg Ala Arg Glu Ser Gln Asp Arg Lys Ile 1745	1750	1755 1760
	Phe Arg Gly Leu Glu Ile Cys Cys Tyr Gly Pro Phe Thr Asn Met Pro 1765	1770	1775
40	Thr Asp Gln Leu Glu Trp Met Val Gln Leu Cys Gly Ala Ser Val Val 1780	1785	1790
	Lys Glu Leu Ser Ser Phe Thr Leu Gly Thr Gly Val His Pro Ile Val 1795	1800	1805
45	Val Val Gln Pro Asp Ala Trp Thr Glu Asp Asn Gly Phe His Ala Ile 1810	1815	1820
50	Gly Gln Met Cys Glu Ala Pro Val Val Thr Arg Glu Trp Val Leu Asp 1825	1830	1835 1840
	Ser Val Ala Leu Tyr Gln Cys Gln Glu Leu Asp Thr Tyr Leu Ile Pro 1845	1850	1855

Gln Ile Pro His Ser His Tyr
1860

- 5 (2) INFORMATION FOR SEQ ID NO:3:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 11283
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- 10 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA to mRNA
- (iii) HYPOTHETICAL: no
- (iv) ANTI-SENSE: no
- (v) ORIGINAL SOURCE
- 15 (A) ORGANISM: Homo sapiens sapiens
- (C) INDIVIDUAL/ISOLATE:
- (D) DEVELOPMENTAL STAGE: adult
- (F) TISSUE TYPE: female breast
- (G) CELL TYPE: normal and cancerous breast cells
- 20 (H) CELL LINE: MCF-7
- (I) ORGANELLE: no
- (vii) IMMEDIATE SOURCE:
- (A) LIBRARY: cDNA library derived from human
- (B) CLONE: obtained using published sequence
- 25 (viii) POSITION IN GENOME:
- (A) CHROMOSOME/SEGMENT: unknown
- (B) MAP POSITION: unknown
- (C) UNITS: unknown
- 30 (ix) FEATURE:
- (A) NAME/KEY: BRCA2
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: gene encoding BRCA2
- 35 protein
- (x) PUBLICATION INFORMATION:
- (A) AUTHORS: Wooster, R. et al.

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(B) TITLE: Identification of the breast cancer
susceptability gene BRCA2

(C) JOURNAL: Nature

(D) VOLUME: 379

5 (E) PAGES: 789-792

(F) DATE: 1995

(K) RELEVANT RESIDUES IN SEQ ID NO:3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

10

ggcggagccg ctgtggcact gctgcgcctc tgctgcgcct cgggtgtctt ttgcggcggg 60

gggtcgccgc cgggagaagc gtgaggggac agatttgtga ccggcgcggg tttgtcagc 120

15

ttactccggc caaaaaagaa ctgcacctct ggagcggact tattaccacaa gcattggagg 180

aatatcgtag gtaaaa 196

20

atg cct att gga tcc aaa gag agg cca aca ttt ttt gaa att ttt aag 244

Met Pro Ile Gly Ser Lys Glu Arg Pro Thr Phe Phe Glu Ile Phe Lys

1 5 10 15

aca cgc tgc aac aaa gca gat tta gga cca ata agt ctt aat tgg ttt 292

Thr Arg Cys Asn Lys Ala Asp Leu Gly Pro Ile Ser Leu Asn Trp Phe

25

20 25 30

gaa gaa ctt tct tca gaa gct cca ccc tat aat tct gaa cct gca gaa 340

Glu Glu Leu Ser Ser Glu Ala Pro Pro Tyr Asn Ser Glu Pro Ala Glu

35 40 45

30

gaa tct gaa cat aaa aac aac aat tac gaa cca aac cta ttt aaa act 388

Glu Ser Glu His Lys Asn Asn Asn Tyr Glu Pro Asn Leu Phe Lys Thr

50 55 60

35

cca caa agg aaa cca tct tat aat cag ctg gct tca act cca ata ata 436

Pro Gln Arg Lys Pro Ser Tyr Asn Gln Leu Ala Ser Thr Pro Ile Ile

65 70 75 80

40

ttc aaa gag caa ggg ctg act ctg ccg ctg tac caa tct cct gta aaa 484

Phe Lys Glu Gln Gly Leu Thr Leu Pro Leu Tyr Gln Ser Pro Val Lys

85 90 95

45

gaa tta gat aaa ttc aaa tta gac tta gga agg aat gtt ccc aat agt 532

Glu Leu Asp Lys Phe Lys Leu Asp Leu Gly Arg Asn Val Pro Asn Ser

100 105 110

aga cat aaa agt ctt cgc aca gtg aaa act aaa atg gat caa gca gat 580

73

	Arg His Lys Ser Leu Arg Thr Val Lys Tyr Lys Met Asp Gln Ala Asp	
	115 120 125	
5	gat gtt tcc tgt cca ctt cta aat tct tgt ctt agt gaa agt cct gtt Asp Val Ser Cys Pro Leu Leu Asn Ser Cys Leu Ser Glu Ser Pro Val	628
	130 135 140	
10	ggt cta caa tgt aca cat gta aca cca caa aga gat aag tca gtg gta Val Leu Gln Cys Thr His Val Thr Pro Gln Arg Asp Lys Ser Val Val	676
	145 150 155 160	
15	tgt ggg agt ttg ttt cat aca cca aag ttt gtg aag ggt cgt cag aca Cys Gly Ser Leu Phe His Thr Pro Lys Phe Val Lys Gly Arg Gln Thr	724
	165 170 175	
	cca aaa cat att tct gaa agt cta gga gct gag gtg gat cct gat atg Pro Lys His Ile Ser Glu Ser Leu Gly Ala Glu Val Asp Pro Asp Met	772
	180 185 190	
20	tct tgg tca agt tct tta gct aca cca ccc acc ctt agt tct act gtg Ser Trp Ser Ser Ser Leu Ala Thr Pro Pro Thr Leu Ser Ser Thr Val	820
	195 200 205	
25	ctc ata gtc aga aat gaa gaa gca tct gaa act gta ttt cct cat gat Leu Ile Val Arg Asn Glu Glu Ala Ser Glu Thr Val Phe Pro His Asp	868
	210 215 220	
30	act act gct aat gtg aaa agc tat ttt tcc aat cat gat gaa agt ctg Thr Thr Ala Asn Val Lys Ser Tyr Phe Ser Asn His Asp Glu Ser Leu	916
	225 230 235 240	
35	aag aaa aat gat aga ttt atc gct tct gtg aca gac agt gaa aac aca Lys Lys Asn Asp Arg Phe Ile Ala Ser Val Thr Asp Ser Glu Asn Thr	964
	245 250 255	
	aat caa aga gaa gct gca agt cat gga ttt gga aaa aca tca ggg aat Asn Gln Arg Glu Ala Ala Ser His Gly Phe Gly Lys Thr Ser Gly Asn	1012
	260 265 270	
40	tca ttt aaa gta aat agc tgc aaa gac cac att gga aag tca atg cca Ser Phe Lys Val Asn Ser Cys Lys Asp His Ile Gly Lys Ser Met Pro	1060
	275 280 285	
45	aat gtc cta gaa gat gaa gta tat gaa aca gtt gta gat acc tct gaa Asn Val Leu Glu Asp Glu Val Tyr Glu Thr Val Val Asp Thr Ser Glu	1108
	290 295 300	
50	gaa gat agt ttt tca tta tgt ttt tct aaa tgt aga aca aaa aat cta Glu Asp Ser Phe Ser Leu Cys Phe Ser Lys Cys Arg Thr Lys Asn Leu	1156
	305 310 315 320	

74

	caa aaa gta aga act agc aag act agg aaa aaa att ttc cat gaa gca Gln Lys Val Arg Thr Ser Lys Thr Arg Lys Lys Ile Phe His Glu Ala 325 330 335	1204
5	aac gct gat gaa tgt gaa aaa tct aaa aac caa gtg aaa gaa aaa tac Asn Ala Asp Glu Cys Glu Lys Ser Lys Asn Gln Val Lys Glu Lys Tyr 340 345 350	1252
10	tca ttt gta tct gaa gtg gaa cca aat gat act gat cca tta gat tca Ser Phe Val Ser Glu Val Glu Pro Asn Asp Thr Asp Pro Leu Asp Ser 355 360 365	1300
15	aat gta gca cat cag aag ccc ttt gag agt gga agt gac aaa atc tcc Asn Val Ala His Gln Lys Pro Phe Glu Ser Gly Ser Asp Lys Ile Ser 370 375 380	1348
20	aag gaa gtt gta ccg tct ttg gcc tgt gaa tgg tct caa cta acc ctt Lys Glu Val Val Pro Ser Leu Ala Cys Glu Trp Ser Gln Leu Thr Leu 385 390 395 400	1396
	tca ggt cta aat gga gcc cag atg gag aaa ata ccc cta ttg cat att Ser Gly Leu Asn Gly Ala Gln Met Glu Lys Ile Pro Leu Leu His Ile 405 410 415	1444
25	tct tca tgt gac caa aat att tca gaa aaa gac cta tta gac aca gag Ser Ser Cys Asp Gln Asn Ile Ser Glu Lys Asp Leu Leu Asp Thr Glu 420 425 430	1492
30	aac aaa aga aag aaa gat ttt ctt act tca gag aat tct ttg cca cgt Asn Lys Arg Lys Lys Asp Phe Leu Thr Ser Glu Asn Ser Leu Pro Arg 435 440 445	1540
35	att tct agc cta cca aaa tca gag aag cca tta aat gag gaa aca gtg Ile Ser Ser Leu Pro Lys Ser Glu Lys Pro Leu Asn Glu Glu Thr Val 450 455 460	1588
40	gta aat aag aga gat gaa gag cag cat ctt gaa tct cat aca gac tgc Val Asn Lys Arg Asp Glu Glu Gln His Leu Glu Ser His Thr Asp Cys 465 470 475 480	1636
	att ctt gca gta aag cag gca ata tct gga act tct cca gtg gct tct Ile Leu Ala Val Lys Gln Ala Ile Ser Gly Thr Ser Pro Val Ala Ser 485 490 495	1684
45	tca ttt cag ggt atc aaa aag tct ata ttc aga ata aga gaa tca cct Ser Phe Gln Gly Ile Lys Lys Ser Ile Phe Arg Ile Arg Glu Ser Pro 500 505 510	1732
50	aaa gag act ttc aat gca agt ttt tca ggt cat atg act gat cca aac Lys Glu Thr Phe Asn Ala Ser Phe Ser Gly His Met Thr Asp Pro Asn 515 520 525	1780

75

	ttt aaa aaa gaa act gaa gcc tct gaa agt gga ctg gaa ata cat act Phe Lys Lys Glu Thr Glu Ala Ser Glu Ser Gly Leu Glu Ile His Thr 530 535 540	1828
5	ggt tgc tca cag aag gag gac tcc tta tgt cca aat tta att gat aat Val Cys Ser Gln Lys Glu Asp Ser Leu Cys Pro Asn Leu Ile Asp Asn 545 550 555 560	1876
10	gga agc tgg cca gcc acc acc aca cag aat tct gta gct ttg aag aat Gly Ser Trp Pro Ala Thr Thr Thr Gln Asn Ser Val Ala Leu Lys Asn 565 570 575	1924
15	gca ggt tta ata tcc act ttg aaa aag aaa aca aat aag ttt att tat Ala Gly Leu Ile Ser Thr Leu Lys Lys Lys Thr Asn Lys Phe Ile Tyr 580 585 590	1972
20	gct ata cat gat gaa aca ttt tat aaa gga aaa aaa ata ccg aaa gac Ala Ile His Asp Glu Thr Phe Tyr Lys Gly Lys Lys Ile Pro Lys Asp 595 600 605	2020
	caa aaa tca gaa cta att aac tgt tca gcc cag ttt gaa gca aat gct Gln Lys Ser Glu Leu Ile Asn Cys Ser Ala Gln Phe Glu Ala Asn Ala 610 615 620	2068
25	ttt gaa gca cca ctt aca ttt gca aat gct gat tca ggt tta ttg cat Phe Glu Ala Pro Leu Thr Phe Ala Asn Ala Asp Ser Gly Leu Leu His 625 630 635 640	2116
30	tct tct gtg aaa aga agc tgt tca cag aat gat tct gaa gaa cca act Ser Ser Val Lys Arg Ser Cys Ser Gln Asn Asp Ser Glu Glu Pro Thr 645 650 655	2164
35	ttg tcc tta act agc tct ttt ggg aca att ctg agg aaa tgt tct aga Leu Ser Leu Thr Ser Ser Phe Gly Thr Ile Leu Arg Lys Cys Ser Arg 660 665 670	2212
40	aat gaa aca tgt tct aat aat aca gta atc tct cag gat ctt gat tat Asn Glu Thr Cys Ser Asn Asn Thr Val Ile Ser Gln Asp Leu Asp Tyr 675 680 685	2260
45	aaa gaa gca aaa tgt aat aag gaa aaa cta cag tta ttt att acc cca Lys Glu Ala Lys Cys Asn Lys Glu Lys Leu Gln Leu Phe Ile Thr Pro 690 695 700	2308
	gaa gct gat tct ctg tca tgc ctg cag gaa gga cag tgt gaa aat gat Glu Ala Asp Ser Leu Ser Cys Leu Gln Glu Gly Gln Cys Glu Asn Asp 705 710 715 720	2356
50	cca aaa agc aaa aaa gtt tca gat ata aaa gaa gag gtc ttg gct gca Pro Lys Ser Lys Lys Val Ser Asp Ile Lys Glu Glu Val Leu Ala Ala 725 730 735	2404

76

	gca tgt cac cca gta caa cat tca aaa gtg gaa tac agt gat act gac Ala Cys His Pro Val Gln His Ser Lys Val Glu Tyr Ser Asp Thr Asp 740 745 750	2452
5	ttt caa tcc cag aaa agt ctt tta tat gat cat gaa aat gcc agc act Phe Gln Ser Gln Lys Ser Leu Leu Tyr Asp His Glu Asn Ala Ser Thr 755 760 765	2500
10	ctt att tta act cct act tcc aag gat gtt ctg tca aac cta gtc atg Leu Ile Leu Thr Pro Thr Ser Lys Asp Val Leu Ser Asn Leu Val Met 770 775 780	2548
15	att tct aga ggc aaa gaa tca tac aaa atg tca gac aag ctc aaa ggt Ile Ser Arg Gly Lys Glu Ser Tyr Lys Met Ser Asp Lys Leu Lys Gly 785 790 795 800	2596
20	aac aat tat gaa tct gat gtt gaa tta acc aaa aat att ccc atg gaa Asn Asn Tyr Glu Ser Asp Val Glu Leu Thr Lys Asn Ile Pro Met Glu 805 810 815	2644
	aag aat caa gat gta tgt gct tta aat gaa aat tat aaa aac gtt gag Lys Asn Gln Asp Val Cys Ala Leu Asn Glu Asn Tyr Lys Asn Val Glu 820 825 830	2692
25	ctg ttg cca cct gaa aaa tac atg aga gta gca tca cct tca aga aag Leu Leu Pro Pro Glu Lys Tyr Met Arg Val Ala Ser Pro Ser Arg Lys 835 840 845	2740
30	gta caa ttc aac caa aac aca aat cta aga gta atc caa aaa aat caa Val Gln Phe Asn Gln Asn Thr Asn Leu Arg Val Ile Gln Lys Asn Gln 850 855 860	2788
35	gaa gaa act act tca att tca aaa ata act gtc aat cca gac tct gaa Glu Glu Thr Thr Ser Ile Ser Lys Ile Thr Val Asn Pro Asp Ser Glu 865 870 875 880	2836
40	gaa ctt ttc tca gac aat gag aat aat ttt gtc ttc caa gta gct aat Glu Leu Phe Ser Asp Asn Glu Asn Asn Phe Val Phe Gln Val Ala Asn 885 890 895	2884
	gaa agg aat aat ctt gct tta gga aat act aag gaa ctt cat gaa aca Glu Arg Asn Asn Leu Ala Leu Gly Asn Thr Lys Glu Leu His Glu Thr 900 905 910	2932
45	gac ttg act tgt gta aac gaa ccc att ttc aag aac tct acc atg gtt Asp Leu Thr Cys Val Asn Glu Pro Ile Phe Lys Asn Ser Thr Met Val 915 920 925	2980
50	tta tat gga gac aca ggt gat aaa caa gca acc caa gtg tca att aaa Leu Tyr Gly Asp Thr Gly Asp Lys Gln Ala Thr Gln Val Ser Ile Lys 930 935 940	3028
	aaa gat ttg gtt tat gtt ctt gca gag gag aac aaa aat agt gta aag	3076

77

	Lys Asp Leu Val Tyr Val Leu Ala Glu Glu Asn Lys Asn Ser Val Lys	
	945 950 955 960	
5	cag cat ata aaa atg act cta ggt caa gat tta aaa tcg gac atc tcc Gln His Ile Lys Met Thr Leu Gly Gln Asp Leu Lys Ser Asp Ile Ser	3124
	965 970 975	
10	ttg aat ata gat aaa ata cca gaa aaa aat aat gat tac atg aac aaa Leu Asn Ile Asp Lys Ile Pro Glu Lys Asn Asn Asp Tyr Met Asn Lys	3172
	980 985 990	
15	tgg gca gga ctc tta ggt cca att tca aat cac agt ttt gga ggt agc Trp Ala Gly Leu Leu Gly Pro Ile Ser Asn His Ser Phe Gly Gly Ser	3220
	995 1000 1005	
	ttc aga aca gct tca aat aag gaa atc aag ctc tct gaa cat aac att Phe Arg Thr Ala Ser Asn Lys Glu Ile Lys Leu Ser Glu His Asn Ile	3268
	1010 1015 1020	
20	aag aag agc aaa atg ttc ttc aaa gat att gaa gaa caa tat cct act Lys Lys Ser Lys Met Phe Phe Lys Asp Ile Glu Glu Gln Tyr Pro Thr	3316
	1025 1030 1035 1040	
25	agt tta gct tgt gtt gaa att gta aat acc ttg gca tta gat aat caa Ser Leu Ala Cys Val Glu Ile Val Asn Thr Leu Ala Leu Asp Asn Gln	3364
	1045 1050 1055	
30	aag aaa ctg agc aag cct cag tca att aat act gta tct gca cat tta Lys Lys Leu Ser Lys Pro Gln Ser Ile Asn Thr Val Ser Ala His Leu	3412
	1060 1065 1070	
35	cag agt agt gta gtt gtt tct gat tgt aaa aat agt cat ata acc cct Gln Ser Ser Val Val Val Ser Asp Cys Lys Asn Ser His Ile Thr Pro	3460
	1075 1080 1085	
40	cag atg tta ttt tcc aag cag gat ttt aat tca aac cat aat tta aca Gln Met Leu Phe Ser Lys Gln Asp Phe Asn Ser Asn His Asn Leu Thr	3508
	1090 1095 1100	
	cct agc caa aag gca gaa att aca gaa ctt tct act ata tta gaa gaa Pro Ser Gln Lys Ala Glu Ile Thr Glu Leu Ser Thr Ile Leu Glu Glu	3556
	1105 1110 1115 1120	
45	tca gga agt cag ttt gaa ttt act cag ttt aga aaa cca agc tac ata Ser Gly Ser Gln Phe Glu Phe Thr Gln Phe Arg Lys Pro Ser Tyr Ile	3604
	1125 1130 1135	
50	ttg cag aag agt aca ttt gaa gtg cct gaa aac cag atg act atc tta Leu Gln Lys Ser Thr Phe Glu Val Pro Glu Asn Gln Met Thr Ile Leu	3652
	1140 1145 1150	
	aag acc act tct gag gaa tgc aga gat gct gat ctt cat gtc ata atg	3700

78

	Lys Thr Thr Ser Glu Glu Cys Arg Asp Ala Asp Leu His Val Ile Met	
	1155 1160 1165	
5	aat gcc cca tcg att ggt cag gta gac agc agc aag caa ttt gaa ggt Asn Ala Pro Ser Ile Gly Gln Val Asp Ser Ser Lys Gln Phe Glu Gly	3748
	1170 1175 1180	
10	aca gtt gaa att aaa cgg aag ttt gct ggc ctg ttg aaa aat gac tgt Thr Val Glu Ile Lys Arg Lys Phe Ala Gly Leu Leu Lys Asn Asp Cys	3796
	1185 1190 1195 1200	
15	aac aaa agt gct tct ggt tat tta aca gat gaa aat gaa gtg ggg ttt Asn Lys Ser Ala Ser Gly Tyr Leu Thr Asp Glu Asn Glu Val Gly Phe	3844
	1205 1210 1215	
	agg ggc ttt tat tct gct cat ggc aca aaa ctg aat gtt tct act gaa Arg Gly Phe Tyr Ser Ala His Gly Thr Lys Leu Asn Val Ser Thr Glu	3892
	1220 1225 1230	
20	gct ctg caa aaa gct gtg aaa ctg ttt agt gat att gag aat att agt Ala Leu Gln Lys Ala Val Lys Leu Phe Ser Asp Ile Glu Asn Ile Ser	3940
	1235 1240 1245	
25	gag gaa act tct gca gag gta cat cca ata agt tta tct tca agt aaa Glu Glu Thr Ser Ala Glu Val His Pro Ile Ser Leu Ser Ser Ser Lys	3988
	1250 1255 1260	
30	tgt cat gat tct gtt gtt tca atg ttt aag ata gaa aat cat aat gat Cys His Asp Ser Val Val Ser Met Phe Lys Ile Glu Asn His Asn Asp	4036
	1265 1270 1275 1280	
35	aaa act gta agt gaa aaa aat aat aaa tgc caa ctg ata tta caa aat Lys Thr Val Ser Glu Lys Asn Asn Lys Cys Gln Leu Ile Leu Gln Asn	4084
	1285 1290 1295	
	aat att gaa atg act act ggc act ttt gtt gaa gaa att act gaa aat Asn Ile Glu Met Thr Thr Gly Thr Phe Val Glu Glu Ile Thr Glu Asn	4132
	1300 1305 1310	
40	tac aag aga aat act gaa aat gaa gat aac aaa tat act gct gcc agt Tyr Lys Arg Asn Thr Glu Asn Glu Asp Asn Lys Tyr Thr Ala Ala Ser	4180
	1315 1320 1325	
45	aga aat tct cat aac tta gaa ttt gat ggc agt gat tca agt aaa aat Arg Asn Ser His Asn Leu Glu Phe Asp Gly Ser Asp Ser Ser Lys Asn	4228
	1330 1335 1340	
50	gat act gtt tgt att cat aaa gat gaa acg gac ttg cta ttt act gat Asp Thr Val Cys Ile His Lys Asp Glu Thr Asp Leu Leu Phe Thr Asp	4276
	1345 1350 1355 1360	
	cag cac aac ata tgt ctt aaa tta tct ggc cag ttt atg aag gag gga Gln His Asn Ile Cys Leu Lys Leu Ser Gly Gln Phe Met Lys Glu Gly	4324

	1365	1370	1375	
5	aac act cag att aaa gaa gat ttg tca gat tta act ttt ttg gaa gtt Asn Thr Gln Ile Lys Glu Asp Leu Ser Asp Leu Thr Phe Leu Glu Val 1380 1385 1390	4372		
10	gcg aaa gct caa gaa gca tgt cat ggt aat act tca aat aaa gaa cag Ala Lys Ala Gln Glu Ala Cys His Gly Asn Thr Ser Asn Lys Glu Gln 1395 1400 1405	4420		
15	ttt act gct act aaa acg gag caa aat ata aaa gat ttt gag act tct Leu Thr Ala Thr Lys Thr Glu Gln Asn Ile Lys Asp Phe Glu Thr Ser 1410 1415 1420	4468		
20	gat aca ttt ttt cag act gca agt ggg aaa aat att agt gtc gcc aaa Asp Thr Phe Phe Gln Thr Ala Ser Gly Lys Asn Ile Ser Val Ala Lys 1425 1430 1435 1440	4516		
25	gag tta ttt aat aaa att gta aat ttc ttt gat cag aaa cca gaa gaa Glu Leu Phe Asn Lys Ile Val Asn Phe Phe Asp Gln Lys Pro Glu Glu 1445 1450 1455	4564		
30	ttg cat aac ttt tcc tta aat tct gaa tta cat tct gac ata aga aag Leu His Asn Phe Ser Leu Asn Ser Glu Leu His Ser Asp Ile Arg Lys 1460 1465 1470	4612		
35	aac aaa atg gac att cta agt tat gag gaa aca gac ata gtt aaa cac Asn Lys Met Asp Ile Leu Ser Tyr Glu Glu Thr Asp Ile Val Lys His 1475 1480 1485	4660		
40	aaa ata ctg aaa gaa agt gtc cca gtt ggt act gga aat caa cta gtg Lys Ile Leu Lys Glu Ser Val Pro Val Gly Thr Gly Asn Gln Leu Val 1490 1495 1500	4708		
45	acc ttc cag gga caa ccc gaa cgt gat gaa aag atc aaa gaa cct act Thr Phe Gln Gly Gln Pro Glu Arg Asp Glu Lys Ile Lys Glu Pro Thr 1505 1510 1515 1520	4756		
50	ctg ttg ggt ttt cat aca gct agc gga aaa aaa gtt aaa att gca aag Leu Leu Gly Phe His Thr Ala Ser Gly Lys Lys Val Lys Ile Ala Lys 1525 1530 1535	4804		
	gaa tct ttg gac aaa gtg aaa aac ctt ttt gat gaa aaa gag caa ggt Glu Ser Leu Asp Lys Val Lys Asn Leu Phe Asp Glu Lys Glu Gln Gly 1540 1545 1550	4852		
	act agt gaa atc acc agt ttt agc cat caa tgg gca aag acc cta aag Thr Ser Glu Ile Thr Ser Phe Ser His Gln Trp Ala Lys Thr Leu Lys 1555 1560 1565	4900		
	tac aga gag gcc tgt aaa gac ctt gaa tta gca tgt gag acc att gag Tyr Arg Glu Ala Cys Lys Asp Leu Glu Leu Ala Cys Glu Thr Ile Glu 1570 1575 1580	4948		

5	atc aca gct gcc cca aag tgt aaa gaa atg cag aat tct ctc aat aat Ile Thr Ala Ala Pro Lys Cys Lys Glu Met Gln Asn Ser Leu Asn Asn 1585 1590 1595 1600	4996
	gat aaa aac ctt gtt tct att gag act gtg gtg cca cct aag ctc tta Asp Lys Asn Leu Val Ser Ile Glu Thr Val Val Pro Pro Lys Leu Leu 1605 1610 1615	5044
10	agt gat aat tta tgt aga caa act gaa aat ctc aaa aca tca aaa agt Ser Asp Asn Leu Cys Arg Gln Thr Glu Asn Leu Lys Thr Ser Lys Ser 1620 1625 1630	5092
15	atc ttt ttg aaa gtt aaa gta cat gaa aat gta gaa aaa gaa aca gca Ile Phe Leu Lys Val Lys Val His Glu Asn Val Glu Lys Glu Thr Ala 1635 1640 1645	5140
20	aaa agt cct gca act tgt tac aca aat cag tcc cct tat tca gtc att Lys Ser Pro Ala Thr Cys Tyr Thr Asn Gln Ser Pro Tyr Ser Val Ile 1650 1655 1660	5188
25	gaa aat tca gcc tta gct ttt tac aca agt tgt agt aga aaa act tct Glu Asn Ser Ala Leu Ala Phe Tyr Thr Ser Cys Ser Arg Lys Thr Ser 1665 1670 1675 1680	5236
	gtg agt cag act tca tta ctt gaa gca aaa aaa tgg ctt aga gaa gga Val Ser Gln Thr Ser Leu Leu Glu Ala Lys Lys Trp Leu Arg Glu Gly 1685 1690 1695	5284
30	ata ttt gat ggt caa cca gaa aga ata aat act gca gat tat gta gga Ile Phe Asp Gly Gln Pro Glu Arg Ile Asn Thr Ala Asp Tyr Val Gly 1700 1705 1710	5332
35	aat tat ttg tat gaa aat aat tca aac agt act ata gct gaa aat gac Asn Tyr Leu Tyr Glu Asn Asn Ser Asn Ser Thr Ile Ala Glu Asn Asp 1715 1720 1725	5380
40	aaa aat cat ctc tcc gaa aaa caa gat act tat tta agt aac agt agc Lys Asn His Leu Ser Glu Lys Gln Asp Thr Tyr Leu Ser Asn Ser Ser 1730 1735 1740	5428
45	atg tct aac agc tat tcc tac cat tct gat gag gta tat aat gat tca Met Ser Asn Ser Tyr Ser Tyr His Ser Asp Glu Val Tyr Asn Asp Ser 1745 1750 1755 1760	5476
	gga tat ctc tca aaa aat aaa ctt gat tct ggt att gag cca gta ttg Gly Tyr Leu Ser Lys Asn Lys Leu Asp Ser Gly Ile Glu Pro Val Leu 1765 1770 1775	5524
50	aag aat gtt gaa gat caa aaa aac act agt ttt tcc aaa gta ata tcc Lys Asn Val Glu Asp Gln Lys Asn Thr Ser Phe Ser Lys Val Ile Ser 1780 1785 1790	5572

	aat gta aaa gat gca aat gca tac cca caa act gta aat gaa gat att Asn Val Lys Asp Ala Asn Ala Tyr Pro Gln Thr Val Asn Glu Asp Ile 1795 1800 1805	5620
5	tgc gtt gag gaa ctt gtg act agc tct tca ccc tgc aaa aat aaa aat Cys Val Glu Glu Leu Val Thr Ser Ser Ser Pro Cys Lys Asn Lys Asn 1810 1815 1820	5668
10	gca gcc att aaa ttg tcc ata tct aat agt aat aat ttt gag gta ggg Ala Ala Ile Lys Leu Ser Ile Ser Asn Ser Asn Asn Phe Glu Val Gly 1825 1830 1835 1840	5716
15	cca cct gca ttt agg ata gcc agt ggt aaa atc cgt ttg tgt tca cat Pro Pro Ala Phe Arg Ile Ala Ser Gly Lys Ile Arg Leu Cys Ser His 1845 1850 1855	5764
20	gaa aca att aaa aaa gtg aaa gac ata ttt aca gac agt ttc agc aaa Glu Thr Ile Lys Lys Val Lys Asp Ile Phe Thr Asp Ser Phe Ser Lys 1860 1865 1870	5812
25	gta att aag gaa aac aac gag aat aaa tca aaa att tgc caa acg aaa Val Ile Lys Glu Asn Asn Glu Asn Lys Ser Lys Ile Cys Gln Thr Lys 1875 1880 1885	5860
30	att atg gca ggt tgt tac gag gca ttg gat gat tca gag gat att ctt Ile Met Ala Gly Cys Tyr Glu Ala Leu Asp Asp Ser Glu Asp Ile Leu 1890 1895 1900	5908
35	cat aac tct cta gat aat gat gaa tgt agc atg cat tca cat aag gtt His Asn Ser Leu Asp Asn Asp Glu Cys Ser Met His Ser His Lys Val 1905 1910 1915 1920	5956
40	ttt gct gac att cag agt gaa gaa att tta caa cat aac caa aat atg Phe Ala Asp Ile Gln Ser Glu Glu Ile Leu Gln His Asn Gln Asn Met 1925 1930 1935	6004
45	tct gga ttg gag aaa gtt tct aaa ata tca cct tgt gat gtt agt ttg Ser Gly Leu Glu Lys Val Ser Lys Ile Ser Pro Cys Asp Val Ser Leu 1940 1945 1950	6052
50	gaa act tca gat ata tgt aaa tgt agt ata ggg aag ctt cat aag tca Glu Thr Ser Asp Ile Cys Lys Cys Ser Ile Gly Lys Leu His Lys Ser 1955 1960 1965	6100
55	gtc tca tct gca aat act tgt ggg att ttt agc aca gca agt gga aaa Val Ser Ser Ala Asn Thr Cys Gly Ile Phe Ser Thr Ala Ser Gly Lys 1970 1975 1980	6148
60	tct gtc cag gta tca gat gct tca tta caa aac gca aga caa gtg ttt Ser Val Gln Val Ser Asp Ala Ser Leu Gln Asn Ala Arg Gln Val Phe 1985 1990 1995 2000	6196
65	tct gaa ata gaa gat agt acc aag caa gtc ttt tcc aaa gta ttg ttt	6244

82

	Ser Glu Ile Glu Asp Ser Thr Lys Gln Val Phe Ser Lys Val Leu Phe	
	2005 2010 2015	
5	aaa agt aac gaa cat tca gac cag ctc aca aga gaa gaa aat act gct Lys Ser Asn Glu His Ser Asp Gln Leu Thr Arg Glu Glu Asn Thr Ala	6292
	2020 2025 2030	
10	ata cgt act cca gaa cat tta ata tcc caa aaa ggc ttt tca tat aat Ile Arg Thr Pro Glu His Leu Ile Ser Gln Lys Gly Phe Ser Tyr Asn	6340
	2035 2040 2045	
15	gtg gta aat tca tct gct ttc tct gga ttt agt aca gca agt gga aag Val Val Asn Ser Ser Ala Phe Ser Gly Phe Ser Thr Ala Ser Gly Lys	6388
	2050 2055 2060	
	caa gtt tcc att tta gaa agt tcc tta cac aaa gtt aag gga gtg tta Gln Val Ser Ile Leu Glu Ser Ser Leu His Lys Val Lys Gly Val Leu	6436
	2065 2070 2075 2080	
20	gag gaa ttt gat tta atc aga act gag cat agt ctt cac tat tca cct Glu Glu Phe Asp Leu Ile Arg Thr Glu His Ser Leu His Tyr Ser Pro	6484
	2085 2090 2095	
25	acg tct aga caa aat gta tca aaa ata ctt cct cgt gtt gat aag aga Thr Ser Arg Gln Asn Val Ser Lys Ile Leu Pro Arg Val Asp Lys Arg	6532
	2100 2105 2110	
30	aac cca gag cac tgt gta aac tca gaa atg gaa aaa acc tgc agt aaa Asn Pro Glu His Cys Val Asn Ser Glu Met Glu Lys Thr Cys Ser Lys	6580
	2115 2120 2125	
35	gaa ttt aaa tta tca aat aac tta aat gtt gaa ggt ggt tct tca gaa Glu Phe Lys Leu Ser Asn Asn Leu Asn Val Glu Gly Gly Ser Ser Glu	6628
	2130 2135 2140	
	aat aat cac tct att aaa gtt tct cca tat ctc tct caa ttt caa caa Asn Asn His Ser Ile Lys Val Ser Pro Tyr Leu Ser Gln Phe Gln Gln	6676
	2145 2150 2155 2160	
40	gac aaa caa cag ttg gta tta gga acc aaa gtc tca ctt gtt gag aac Asp Lys Gln Gln Leu Val Leu Gly Thr Lys Val Ser Leu Val Glu Asn	6724
	2165 2170 2175	
45	att cat gtt ttg gga aaa gaa cag gct tca cct aaa aac gta aaa atg Ile His Val Leu Gly Lys Glu Gln Ala Ser Pro Lys Asn Val Lys Met	6772
	2180 2185 2190	
50	gaa att ggt aaa act gaa act ttt tct gat gtt cct gtg aaa aca aat Glu Ile Gly Lys Thr Glu Thr Phe Ser Asp Val Pro Val Lys Thr Asn	6820
	2195 2200 2205	
	ata gaa gtt tgt tct act tac tcc aaa gat tca gaa aac tac ttt gaa Ile Glu Val Cys Ser Thr Tyr Ser Lys Asp Ser Glu Asn Tyr Phe Glu	6868

	2210	2215	2220	
5	aca gaa gca gta gaa att gct aaa gct ttt atg gaa gat gat gaa ctg Thr Glu Ala Val Glu Ile Ala Lys Ala Phe Met Glu Asp Asp Glu Leu 2225 2230 2235 2240	6916		
10	aca gat tct aaa ctg cca agt cat gcc aca cat tct ctt ttt aca tgt Thr Asp Ser Lys Leu Pro Ser His Ala Thr His Ser Leu Phe Thr Cys 2245 2250 2255	6964		
15	ccc gaa aat gag gaa atg gtt ttg tca aat tca aga att gga aaa aga Pro Glu Asn Glu Glu Met Val Leu Ser Asn Ser Arg Ile Gly Lys Arg 2260 2265 2270	7012		
	aga gga gag ccc ctt atc tta gtg gga gaa ccc tca atc aaa aga aac Arg Gly Glu Pro Leu Ile Leu Val Gly Glu Pro Ser Ile Lys Arg Asn 2275 2280 2285	7060		
20	tta tta aat gaa ttt gac agg ata ata gaa aat caa gaa aaa tcc tta Leu Leu Asn Glu Phe Asp Arg Ile Ile Glu Asn Gln Glu Lys Ser Leu 2290 2295 2300	7108		
25	aag gct tca aaa agc act cca gat ggc aca ata aaa gat cga aga ttg Lys Ala Ser Lys Ser Thr Pro Asp Gly Thr Ile Lys Asp Arg Arg Leu 2305 2310 2315 2320	7156		
30	ttt atg cat cat gtt tct tta gag cag att acc tgt gta ccc ttt cgc Phe Met His His Val Ser Leu Glu Pro Ile Thr Cys Val Pro Phe Arg 2325 2330 2335	7204		
	aca act aag gaa cgt caa gag ata cag aat cca aat ttt acc gca cct Thr Thr Lys Glu Arg Gln Glu Ile Gln Asn Pro Asn Phe Thr Ala Pro 2340 2345 2350	7252		
35	ggt caa gaa ttt ctg tct aaa tct cat ttg tat gaa cat ctg act ttg Gly Gln Glu Phe Leu Ser Lys Ser His Leu Tyr Glu His Leu Thr Leu 2355 2360 2365	7300		
40	gaa aaa tct tca agc aat tta gca gtt tca gga cat cca ttt tat caa Glu Lys Ser Ser Ser Asn Leu Ala Val Ser Gly His Pro Phe Tyr Gln 2370 2375 2380	7348		
45	gtt tct gct aca aga aat gaa aaa atg aga cac ttg att act aca ggc Val Ser Ala Thr Arg Asn Glu Lys Met Arg His Leu Ile Thr Thr Gly 2385 2390 2395 2400	7396		
50	aga cca acc aaa gtc ttt gtt cca cct ttt aaa act aaa tca cat ttt Arg Pro Thr Lys Val Phe Val Pro Pro Phe Lys Thr Lys Ser His Phe 2405 2410 2415	7444		
	cac aga gtt gaa cag tgt gtt agg aat att aac ttg gag gaa aac aga His Arg Val Glu Gln Cys Val Arg Asn Ile Asn Leu Glu Glu Asn Arg	7492		

	2420	2425	2430	
5	caa aag caa aac att gat gga cat ggc tct gat gat agt aaa aat aag Gln Lys Gln Asn Ile Asp Gly His Gly Ser Asp Asp Ser Lys Asn Lys 2435 2440 2445	7540		
10	att aat gac aat gag att cat cag ttt aac aaa aac aac tcc aat caa Ile Asn Asp Asn Glu Ile His Gln Phe Asn Lys Asn Asn Ser Asn Gln 2450 2455 2460	7588		
15	gca gca gct gta act ttc aca aag tgt gaa gaa gaa cct tta gat tta Ala Ala Ala Val Thr Phe Thr Lys Cys Glu Glu Glu Pro Leu Asp Leu 2465 2470 2475 2480	7636		
20	att aca agt ctt cag aat gcc aga gat ata cag gat atg cga att aag Ile Thr Ser Leu Gln Asn Ala Arg Asp Ile Gln Asp Met Arg Ile Lys 2485 2490 2495	7684		
25	aag aaa caa agg caa cgc gtc ttt cca cag cca ggc agt ctg tat ctt Lys Lys Gln Arg Gln Arg Val Phe Pro Gln Pro Gly Ser Leu Tyr Leu 2500 2505 2510	7732		
30	gca aaa aca tcc act ctg cct cga atc tct ctg aaa gca gca gta gga Ala Lys Thr Ser Thr Leu Pro Arg Ile Ser Leu Lys Ala Ala Val Gly 2515 2520 2525	7780		
35	ggc caa gtt ccc tct gcg tgt tct cat aaa cag ctg tat acg tat ggc Gly Gln Val Pro Ser Ala Cys Ser His Lys Gln Leu Tyr Thr Tyr Gly 2530 2535 2540	7828		
40	gtt tct aaa cat tgc ata aaa att aac agc aaa aat gca gag tct ttt Val Ser Lys His Cys Ile Lys Ile Asn Ser Lys Asn Ala Glu Ser Phe 2545 2550 2555 2560	7876		
45	cag ttt cac act gaa gat tat ttt ggt aag gaa agt tta tgg act gga Gln Phe His Thr Glu Asp Tyr Phe Gly Lys Glu Ser Leu Trp Thr Gly 2565 2570 2575	7924		
50	aaa gga ata cag ttg gct gat ggt gga tgg ctc ata ccc tcc aat gat Lys Gly Ile Gln Leu Ala Asp Gly Gly Trp Leu Ile Pro Ser Asn Asp 2580 2585 2590	7972		
	gga aag gct gga aaa gaa gaa ttt tat agg gct ctg tgt gac act cca Gly Lys Ala Gly Lys Glu Glu Phe Tyr Arg Ala Leu Cys Asp Thr Pro 2595 2600 2605	8020		
	ggc gtg gat cca aag ctt att tct aga att tgg gtt tat aat cac tat Gly Val Asp Pro Lys Leu Ile Ser Arg Ile Trp Val Tyr Asn His Tyr 2610 2615 2620	8068		
	aga tgg atc ata tgg aaa ctg gca gct atg gaa tgt gcc ttt cct aag Arg Trp Ile Ile Trp Lys Leu Ala Ala Met Glu Cys Ala Phe Pro Lys	8116		

85

	2625	2630	2635	2640	
	gaa ttt gct aat aga tgc cta agc cca gaa agg gtg ctt ctt caa cta				8164
	Glu Phe Ala Asn Arg Cys Leu Ser Pro Glu Arg Val Leu Leu Gln Leu				
5	2645	2650	2655		
	aaa tac aga tat gat acg gaa att gat aga agc aga aga tcg gct ata				8212
	Lys Tyr Arg Tyr Asp Thr Glu Ile Asp Arg Ser Arg Arg Ser Ala Ile				
	2660	2665	2670		
10	aaa aag ata atg gaa agg gat gac aca gct gca aaa aca ctt gtt ctc				8260
	Lys Lys Ile Met Glu Arg Asp Asp Thr Ala Ala Lys Thr Leu Val Leu				
	2675	2680	2685		
15	tgt gtt tct gac ata att tca ttg agc gca aat ata tct gaa act tct				8308
	Cys Val Ser Asp Ile Ile Ser Leu Ser Ala Asn Ile Ser Glu Thr Ser				
	2690	2695	2700		
20	agc aat aaa act agt agt gca gat acc caa aaa gtg gcc att att gaa				8356
	Ser Asn Lys Thr Ser Ser Ala Asp Thr Gln Lys Val Ala Ile Ile Glu				
	2705	2710	2715	2720	
25	ctt aca gat ggg tgg tat gct gtt aag gcc cag tta gat cct ccc ctc				8404
	Leu Thr Asp Gly Trp Tyr Ala Val Lys Ala Gln Leu Asp Pro Pro Leu				
	2725	2730	2735		
30	tta gct gtc tta aag aat ggc aga ctg aca gtt ggt cag aag att att				8452
	Leu Ala Val Leu Lys Asn Gly Arg Leu Thr Val Gly Gln Lys Ile Ile				
	2740	2745	2750		
35	ctt cat gga gca gaa ctg gtg ggc tct cct gat gcc tgt aca cct ctt				8500
	Leu His Gly Ala Glu Leu Val Gly Ser Pro Asp Ala Cys Thr Pro Leu				
	2755	2760	2765		
40	gaa gcc cca gaa tct ctt atg tta aag att tct gct aac agt act cgg				8548
	Glu Ala Pro Glu Ser Leu Met Leu Lys Ile Ser Ala Asn Ser Thr Arg				
	2770	2775	2780		
45	cct gct cgc tgg tat acc aaa ctt gga ttc ttt cct gac cct aga cct				8596
	Pro Ala Arg Trp Tyr Thr Lys Leu Gly Phe Phe Pro Asp Pro Arg Pro				
	2785	2790	2795	2800	
50	ttt cct ctg ccc tta tca tcg ctt ttc agt gat gga gga aat gtt ggt				8644
	Phe Pro Leu Pro Leu Ser Ser Leu Phe Ser Asp Gly Gly Asn Val Gly				
	2805	2810	2815		
50	tgt gtt gat gta att att caa aga gca tac cct ata cag cgg atg gag				8692
	Cys Val Asp Val Ile Ile Gln Arg Ala Tyr Pro Ile Gln Arg Met Glu				
	2820	2825	2830		
	aag aca tca tct gga tta tac ata ttt cgc aat gaa aga gag gaa gaa				8740
	Lys Thr Ser Ser Gly Leu Tyr Ile Phe Arg Asn Glu Arg Glu Glu Glu				

86

	2835	2840	2845	
5	aag gaa gca gca aaa tat gtg gag gcc caa caa aag aga cta gaa gcc Lys Glu Ala Ala Lys Tyr Val Glu Ala Gln Gln Lys Arg Leu Glu Ala 2850 2855 2860	8788		
10	tta ttc act aaa att cag gag gaa ttt gaa gaa cat gaa gaa aac aca Leu Phe Thr Lys Ile Gln Glu Glu Phe Glu Glu His Glu Glu Asn Thr 2865 2870 2875 2880	8836		
15	aca aaa cca tat tta cca tca cgt gca cta aca aga cag caa gtt cgt Thr Lys Pro Tyr Leu Pro Ser Arg Ala Leu Thr Arg Gln Gln Val Arg 2885 2890 2895	8884		
20	gct ttg caa gat ggt gca gag ctt tat gaa gca gtg aag aat gca gca Ala Leu Gln Asp Gly Ala Glu Leu Tyr Glu Ala Val Lys Asn Ala Ala 2900 2905 2910	8932		
25	gac cca gct tac ctt gag ggt tat ttc agt gaa gag cag tta aga gcc Asp Pro Ala Tyr Leu Glu Gly Tyr Phe Ser Glu Glu Gln Leu Arg Ala 2915 2920 2925	8980		
30	ttg aat aat cac agg caa atg ttg aat gat aag aaa caa gct cag atc Leu Asn Asn His Arg Gln Met Leu Asn Asp Lys Lys Gln Ala Gln Ile 2930 2935 2940	9028		
35	cag ttg gaa att agg aag gcc atg gaa tct gct gaa caa aag gaa caa Gln Leu Glu Ile Arg Lys Ala Met Glu Ser Ala Glu Gln Lys Glu Gln 2945 2950 2955 2960	9076		
40	ggt tta tca agg gat gtc aca acc gtg tgg aag ttg cgt att gta agc Gly Leu Ser Arg Asp Val Thr Thr Val Trp Lys Leu Arg Ile Val Ser 2965 2970 2975	9124		
45	tat tca aaa aaa gaa aaa gat tca gtt ata ctg agt att tgg cgt cca Tyr Ser Lys Lys Glu Lys Asp Ser Val Ile Leu Ser Ile Trp Arg Pro 2980 2985 2990	9172		
50	tca tca gat tta tat tct ctg tta aca gaa gga aag aga tac aga att Ser Ser Asp Leu Tyr Ser Leu Leu Thr Glu Gly Lys Arg Tyr Arg Ile 2995 3000 3005	9220		
	tat cat ctt gca act tca aaa tct aaa agt aaa tct gaa aga gct aac Tyr His Leu Ala Thr Ser Lys Ser Lys Ser Lys Ser Glu Arg Ala Asn 3010 3015 3020	9268		
	ata cag tta gca gcg aca aaa aaa act cag tat caa caa cta ccg gtt Ile Gln Leu Ala Ala Thr Lys Lys Thr Gln Tyr Gln Gln Leu Pro Val 3025 3030 3035 3040	9316		
	tca gat gaa att tta ttt cag att tac cag cca cgg gag ccc ctt cac	9364		

87

	Ser Asp Glu Ile Leu Phe Gln Ile Tyr Gln Pro Arg Glu Pro Leu His	
	3045 3050 3055	
5	ttc agc aaa ttt tta gat cca gac ttt cag cca tct tgt tct gag gtg Phe Ser Lys Phe Leu Asp Pro Asp Phe Gln Pro Ser Cys Ser Glu Val	9412
	3060 3065 3070	
10	gac cta ata gga ttt gtc gtt tct gtt gtg aaa aaa aca gga ctt gcc Asp Leu Ile Gly Phe Val Val Ser Val Val Lys Lys Thr Gly Leu Ala	9460
	3075 3080 3085	
15	cct ttc gtc tat ttg tca gac gaa tgt tac aat tta ctg gca ata aag Pro Phe Val Tyr Leu Ser Asp Glu Cys Tyr Asn Leu Leu Ala Ile Lys	9508
	3090 3095 3100	
20	ttt tgg ata gac ctt aat gag gac att att aag cct cat atg tta att Phe Trp Ile Asp Leu Asn Glu Asp Ile Ile Lys Pro His Met Leu Ile	9556
	3105 3110 3115 3120	
25	gct gca agc aac ctc cag tgg cga cca gaa tcc aaa tca ggc ctt ctt Ala Ala Ser Asn Leu Gln Trp Arg Pro Glu Ser Lys Ser Gly Leu Leu	9604
	3125 3130 3135	
30	act tta ttt gct gga gat ttt tct gtg ttt tct gct agt cca aaa gag Thr Leu Phe Ala Gly Asp Phe Ser Val Phe Ser Ala Ser Pro Lys Glu	9652
	3140 3145 3150	
35	ggc cac ttt caa gag aca ttc aac aaa atg aaa aat act gtt gag aat Gly His Phe Gln Glu Thr Phe Asn Lys Met Lys Asn Thr Val Glu Asn	9700
	3155 3160 3165	
40	att gac ata ctt tgc aat gaa gca gaa aac aag ctt atg cat ata ctg Ile Asp Ile Leu Cys Asn Glu Ala Glu Asn Lys Leu Met His Ile Leu	9748
	3170 3175 3180	
45	cat gca aat gat ccc aag tgg tcc acc cca act aaa gac tgt act tca His Ala Asn Asp Pro Lys Trp Ser Thr Pro Thr Lys Asp Cys Thr Ser	9796
	3185 3190 3195 3200	
50	ggg ccg tac act gct caa atc att cct ggt aca gga aac aag ctt ctg Gly Pro Tyr Thr Ala Gln Ile Ile Pro Gly Thr Gly Asn Lys Leu Leu	9844
	3205 3210 3215	
55	atg tct tct cct aat tgt gag ata tat tat caa agt cct tta tca ctt Met Ser Ser Pro Asn Cys Glu Ile Tyr Tyr Gln Ser Pro Leu Ser Leu	9892
	3220 3225 3230	
60	tgt atg gcc aaa agg aag tct gtt tcc aca cct gtc tca gcc cag atg Cys Met Ala Lys Arg Lys Ser Val Ser Thr Pro Val Ser Ala Gln Met	9940
	3235 3240 3245	
65	act tca aag tct tgt aaa ggg gag aaa gag att gat gac caa aag aac Thr Ser Lys Ser Cys Lys Gly Glu Lys Glu Ile Asp Asp Gln Lys Asn	9988

88

	3250	3255	3260	
5	tgc aaa aag aga aga gcc ttg gat ttc ttg agt aga ctg cct tta cct Cys Lys Lys Arg Arg Ala Leu Asp Phe Leu Ser Arg Leu Pro Leu Pro 3265 3270 3275 3280			10036
10	cca cct gtt agt ccc att tgt aca ttt gtt tct ccg gct gca cag aag Pro Pro Val Ser Pro Ile Cys Thr Phe Val Ser Pro Ala Ala Gln Lys 3285 3290 3295			10084
15	gca ttt cag cca cca agg agt tgt ggc acc aaa tac gaa aca ccc ata Ala Phe Gln Pro Pro Arg Ser Cys Gly Thr Lys Tyr Glu Thr Pro Ile 3300 3305 3310			10132
20	aag aaa aaa gaa ctg aat tct cct cag atg act cca ttt aaa aaa ttc Lys Lys Lys Glu Leu Asn Ser Pro Gln Met Thr Pro Phe Lys Lys Phe 3315 3320 3325			10180
25	aat gaa att tct ctt ttg gaa agt aat tca ata gct gac gaa gaa ctt Asn Glu Ile Ser Leu Leu Glu Ser Asn Ser Ile Ala Asp Glu Glu Leu 3330 3335 3340			10228
30	gca ttg ata aat acc caa gct ctt ttg tct ggt tca aca gga gaa aaa Ala Leu Ile Asn Thr Gln Ala Leu Leu Ser Gly Ser Thr Gly Glu Lys 3345 3350 3355 3360			10276
35	caa ttt ata tct gtc agt gaa tcc act agg act gct ccc acc agt tca Gln Phe Ile Ser Val Ser Glu Ser Thr Arg Thr Ala Pro Thr Ser Ser 3365 3370 3375			10324
40	gaa gat tat ctc aga ctg aaa cga cgt tgt act aca tct ctg atc aaa Glu Asp Tyr Leu Arg Leu Lys Arg Arg Cys Thr Thr Ser Leu Ile Lys 3380 3385 3390			10372
45	gaa cag gag agt tcc cag gcc agt acg gaa gaa tgt gag aaa aat aag Glu Gln Glu Ser Ser Gln Ala Ser Thr Glu Glu Cys Glu Lys Asn Lys 3395 3400 3405			10420
50	cag gac aca att aca act aaa aaa tat atc taagcatttg caaaggcgac Gln Asp Thr Ile Thr Thr Lys Lys Tyr Ile 3410 3415			10470
	aataaattat tgacgcttaa cctttccagt ttataagact ggaatataat ttcaaacccac			10530
	acattagtac ttatgttgcm caatgagaaa agaaattagt ttcaaattta cctcagcgtt			10590
	tgtgtatcgg gcaaaaaatcg ttttgccga ttccgtattg gtatactttt gcctcagttg			10650
	catatcctaa aactaaatgt aatttattaa ctaatcaaga aaaacatctt tggctgagct			10710

5 cggtaggtca tgcctgtaat cccaacactt tgagaagctg aggtggggagg agtgcttgag 10770
 gccaggaggt caagaccagc ctgggcaaca tagggagacc ccatctttac gaagaaaaaa 10830
 10 aaaaagggga aaagaaaatc ttttaaatct ttggatttca ctacaagtat tattttacaa 10890
 gtgaaataaa cataccattt tcttttagat tgtgtcatta aatggaatga ggtctcttag 10950
 15 tacagttatt ttgatgcaga taattccttt tagtttagct actattttag gggatttttt 11010
 ttagaggtaa ctactatga aatagttccc cttaatgcaa atatgttggg tctgcaatag 11070
 20 ttccatctcg ttcaaaaatc rgggtgaawa tgaagagtg tgtyccttt tgagcaattc 11130
 tcctccttaa gtcagcrtga ttataagaa aatagaaccc ycagtgtaac yctaattcct 11190
 25 ttttctatt ccagtgtgat ctctgaakt aaattacttc mactaaaaat tcaaaaactt 11250
 30 waamtacgaa rawttcawag twgatttatt ttt 11283

35

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3418
 (B) TYPE: amino acid
 40 (C) STRANDEDNESS: single
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

45 (v) ORIGINAL SOURCE

(A) ORGANISM: Homo sapiens sapiens

(C) INDIVIDUAL/ISOLATE:

90

(D) DEVELOPMENTAL STAGE: adult

(F) TISSUE TYPE: female breast

(G) CELL TYPE: normal breast tissue

(H) CELL LINE: HMEC

5 (I) ORGANELLE: no

(ix) FEATURE:

(A) NAME/KEY: BRCA2 protein

(B) LOCATION: 1 to 3418; Genbank locus HSU43746

(C) IDENTIFICATION METHOD:

10 (D) OTHER INFORMATION: BRCA2 protein has a
negative regulatory effect on growth of human mammary cells.

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Wooster, R. et al.

15 (B) TITLE: Identification of the breast cancer
susceptibility gene BRCA2

(C) JOURNAL: Nature

(D) VOLUME: 379

(E) PAGES: 789-792

(F) DATE: 1995

20 (K) RELEVANT RESIDUES IN SEQ ID NO:4: granin
box domain at amino acids 3334-3344

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

25 Met Pro Ile Gly Ser Lys Glu Arg Pro Thr Phe Phe Glu Ile Phe Lys
1 5 10 1530 Thr Arg Cys Asn Lys Ala Asp Leu Gly Pro Ile Ser Leu Asn Trp Phe
20 25 3035 Glu Glu Leu Ser Ser Glu Ala Pro Pro Tyr Asn Ser Glu Pro Ala Glu
35 40 4540 Glu Ser Glu His Lys Asn Asn Asn Tyr Glu Pro Asn Leu Phe Lys Thr
50 55 60Pro Gln Arg Lys Pro Ser Tyr Asn Gln Leu Ala Ser Thr Pro Ile Ile
65 70 75 8040 Phe Lys Glu Gln Gly Leu Thr Leu Pro Leu Tyr Gln Ser Pro Val Lys
85 90 95

91

	Glu Leu Asp Lys Phe Lys Leu Asp Leu Gly Arg Asn Val Pro Asn Ser
	100 105 110
5	Arg His Lys Ser Leu Arg Thr Val Lys Tyr Lys Met Asp Gln Ala Asp
	115 120 125
	Asp Val Ser Cys Pro Leu Leu Asn Ser Cys Leu Ser Glu Ser Pro Val
	130 135 140
10	Val Leu Gln Cys Thr His Val Thr Pro Gln Arg Asp Lys Ser Val Val
	145 150 155 160
	Cys Gly Ser Leu Phe His Thr Pro Lys Phe Val Lys Gly Arg Gln Thr
	165 170 175
15	Pro Lys His Ile Ser Glu Ser Leu Gly Ala Glu Val Asp Pro Asp Met
	180 185 190
	Ser Trp Ser Ser Ser Leu Ala Thr Pro Pro Thr Leu Ser Ser Thr Val
20	195 200 205
	Leu Ile Val Arg Asn Glu Glu Ala Ser Glu Thr Val Phe Pro His Asp
	210 215 220
25	Thr Thr Ala Asn Val Lys Ser Tyr Phe Ser Asn His Asp Glu Ser Leu
	225 230 235 240
	Lys Lys Asn Asp Arg Phe Ile Ala Ser Val Thr Asp Ser Glu Asn Thr
30	245 250 255
	Asn Gln Arg Glu Ala Ala Ser His Gly Phe Gly Lys Thr Ser Gly Asn
	260 265 270
35	Ser Phe Lys Val Asn Ser Cys Lys Asp His Ile Gly Lys Ser Met Pro
	275 280 285
40	Asn Val Leu Glu Asp Glu Val Tyr Glu Thr Val Val Asp Thr Ser Glu
	290 295 300
	Glu Asp Ser Phe Ser Leu Cys Phe Ser Lys Cys Arg Thr Lys Asn Leu
45	305 310 315 320
	Gln Lys Val Arg Thr Ser Lys Thr Arg Lys Lys Ile Phe His Glu Ala
	325 330 335
50	Asn Ala Asp Glu Cys Glu Lys Ser Lys Asn Gln Val Lys Glu Lys Tyr
	340 345 350

92

	Ser Phe Val Ser Glu Val Glu Pro Asn Asp Thr Asp Pro Leu Asp Ser
	355 360 365
5	Asn Val Ala His Gln Lys Pro Phe Glu Ser Gly Ser Asp Lys Ile Ser
	370 375 380
	Lys Glu Val Val Pro Ser Leu Ala Cys Glu Trp Ser Gln Leu Thr Leu
	385 390 395 400
10	Ser Gly Leu Asn Gly Ala Gln Met Glu Lys Ile Pro Leu Leu His Ile
	405 410 415
	Ser Ser Cys Asp Gln Asn Ile Ser Glu Lys Asp Leu Leu Asp Thr Glu
15	420 425 430
	Asn Lys Arg Lys Lys Asp Phe Leu Thr Ser Glu Asn Ser Leu Pro Arg
	435 440 445
20	Ile Ser Ser Leu Pro Lys Ser Glu Lys Pro Leu Asn Glu Glu Thr Val
	450 455 460
	Val Asn Lys Arg Asp Glu Glu Gln His Leu Glu Ser His Thr Asp Cys
	465 470 475 480
25	Ile Leu Ala Val Lys Gln Ala Ile Ser Gly Thr Ser Pro Val Ala Ser
	485 490 495
30	Ser Phe Gln Gly Ile Lys Lys Ser Ile Phe Arg Ile Arg Glu Ser Pro
	500 505 510
35	Lys Glu Thr Phe Asn Ala Ser Phe Ser Gly His Met Thr Asp Pro Asn
	515 520 525
40	Phe Lys Lys Glu Thr Glu Ala Ser Glu Ser Gly Leu Glu Ile His Thr
	530 535 540
	Val Cys Ser Gln Lys Glu Asp Ser Leu Cys Pro Asn Leu Ile Asp Asn
	545 550 555 560
45	Gly Ser Trp Pro Ala Thr Thr Thr Gln Asn Ser Val Ala Leu Lys Asn
	565 570 575
50	Ala Gly Leu Ile Ser Thr Leu Lys Lys Lys Thr Asn Lys Phe Ile Tyr
	580 585 590
	Ala Ile His Asp Glu Thr Phe Tyr Lys Gly Lys Lys Ile Pro Lys Asp

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	595	600	605
	Gln Lys Ser Glu Leu Ile Asn Cys Ser Ala Gln Phe Glu Ala Asn Ala		
	610	615	620
5			
	Phe Glu Ala Pro Leu Thr Phe Ala Asn Ala Asp Ser Gly Leu Leu His		
	625	630	635 640
10			
	Ser Ser Val Lys Arg Ser Cys Ser Gln Asn Asp Ser Glu Glu Pro Thr		
	645	650	655
15			
	Leu Ser Leu Thr Ser Ser Phe Gly Thr Ile Leu Arg Lys Cys Ser Arg		
	660	665	670
20			
	Asn Glu Thr Cys Ser Asn Asn Thr Val Ile Ser Gln Asp Leu Asp Tyr		
	675	680	685
25			
	Lys Glu Ala Lys Cys Asn Lys Glu Lys Leu Gln Leu Phe Ile Thr Pro		
	690	695	700
30			
	Glu Ala Asp Ser Leu Ser Cys Leu Gln Glu Gly Gln Cys Glu Asn Asp		
	705	710	715 720
35			
	Pro Lys Ser Lys Lys Val Ser Asp Ile Lys Glu Glu Val Leu Ala Ala		
	725	730	735
40			
	Ala Cys His Pro Val Gln His Ser Lys Val Glu Tyr Ser Asp Thr Asp		
	740	745	750
45			
	Phe Gln Ser Gln Lys Ser Leu Leu Tyr Asp His Glu Asn Ala Ser Thr		
	755	760	765
50			
	Leu Ile Leu Thr Pro Thr Ser Lys Asp Val Leu Ser Asn Leu Val Met		
	770	775	780
	Ile Ser Arg Gly Lys Glu Ser Tyr Lys Met Ser Asp Lys Leu Lys Gly		
	785	790	795 800
	Asn Asn Tyr Glu Ser Asp Val Glu Leu Thr Lys Asn Ile Pro Met Glu		
	805	810	815

5 Lys Asn Gln Asp Val Cys Ala Leu Asn Glu Asn Tyr Lys Asn Val Glu
820 825 830

Leu Leu Pro Pro Glu Lys Tyr Met Arg Val Ala Ser Pro Ser Arg Lys
835 840 845

10 Val Gln Phe Asn Gln Asn Thr Asn Leu Arg Val Ile Gln Lys Asn Gln
850 855 860

15 Glu Glu Thr Thr Ser Ile Ser Lys Ile Thr Val Asn Pro Asp Ser Glu
865 870 875 880

20 Glu Leu Phe Ser Asp Asn Glu Asn Asn Phe Val Phe Gln Val Ala Asn
885 890 895

Glu Arg Asn Asn Leu Ala Leu Gly Asn Thr Lys Glu Leu His Glu Thr
900 905 910

25 Asp Leu Thr Cys Val Asn Glu Pro Ile Phe Lys Asn Ser Thr Met Val
915 920 925

30 Leu Tyr Gly Asp Thr Gly Asp Lys Gln Ala Thr Gln Val Ser Ile Lys
930 935 940

35 Lys Asp Leu Val Tyr Val Leu Ala Glu Glu Asn Lys Asn Ser Val Lys
945 950 955 960

40 Gln His Ile Lys Met Thr Leu Gly Gln Asp Leu Lys Ser Asp Ile Ser
965 970 975

Leu Asn Ile Asp Lys Ile Pro Glu Lys Asn Asn Asp Tyr Met Asn Lys
980 985 990

45 Trp Ala Gly Leu Leu Gly Pro Ile Ser Asn His Ser Phe Gly Gly Ser
995 1000 1005

50 Phe Arg Thr Ala Ser Asn Lys Glu Ile Lys Leu Ser Glu His Asn Ile
1010 1015 1020

95

5 Lys Lys Ser Lys Met Phe Phe Lys Asp Ile Glu Glu Gln Tyr Pro Thr
1025 1030 1035 1040

10 Ser Leu Ala Cys Val Glu Ile Val Asn Thr Leu Ala Leu Asp Asn Gln
1045 1050 1055

15 Gln Ser Ser Val Val Val Ser Asp Cys Lys Asn Ser His Ile Thr Pro
1075 1080 1085

20 Gln Met Leu Phe Ser Lys Gln Asp Phe Asn Ser Asn His Asn Leu Thr
1090 1095 1100

25 Pro Ser Gln Lys Ala Glu Ile Thr Glu Leu Ser Thr Ile Leu Glu Glu
1105 1110 1115 1120

30 Ser Gly Ser Gln Phe Glu Phe Thr Gln Phe Arg Lys Pro Ser Tyr Ile
1125 1130 1135

35 Leu Gln Lys Ser Thr Phe Glu Val Pro Glu Asn Gln Met Thr Ile Leu
1140 1145 1150

40 Lys Thr Thr Ser Glu Glu Cys Arg Asp Ala Asp Leu His Val Ile Met
1155 1160 1165

45 Asn Ala Pro Ser Ile Gly Gln Val Asp Ser Ser Lys Gln Phe Glu Gly
1170 1175 1180

50 Thr Val Glu Ile Lys Arg Lys Phe Ala Gly Leu Leu Lys Asn Asp Cys
1185 1190 1195 1200

Asn Lys Ser Ala Ser Gly Tyr Leu Thr Asp Glu Asn Glu Val Gly Phe
1205 1210 1215

Arg Gly Phe Tyr Ser Ala His Gly Thr Lys Leu Asn Val Ser Thr Glu
1220 1225 1230

96

	Ala Leu Gln Lys Ala Val Lys Leu Phe Ser Asp Ile Glu Asn Ile Ser
	1235 1240 1245
5	Glu Glu Thr Ser Ala Glu Val His Pro Ile Ser Leu Ser Ser Ser Lys
	1250 1255 1260
10	Cys His Asp Ser Val Val Ser Met Phe Lys Ile Glu Asn His Asn Asp
	1265 1270 1275 1280
15	Lys Thr Val Ser Glu Lys Asn Asn Lys Cys Gln Leu Ile Leu Gln Asn
	1285 1290 1295
20	Asn Ile Glu Met Thr Thr Gly Thr Phe Val Glu Glu Ile Thr Glu Asn
	1300 1305 1310
	Tyr Lys Arg Asn Thr Glu Asn Glu Asp Asn Lys Tyr Thr Ala Ala Ser
	1315 1320 1325
25	Arg Asn Ser His Asn Leu Glu Phe Asp Gly Ser Asp Ser Ser Lys Asn
	1330 1335 1340
30	Asp Thr Val Cys Ile His Lys Asp Glu Thr Asp Leu Leu Phe Thr Asp
	1345 1350 1355 1360
35	Gln His Asn Ile Cys Leu Lys Leu Ser Gly Gln Phe Met Lys Glu Gly
	1365 1370 1375
40	Asn Thr Gln Ile Lys Glu Asp Leu Ser Asp Leu Thr Phe Leu Glu Val
	1380 1385 1390
45	Ala Lys Ala Gln Glu Ala Cys His Gly Asn Thr Ser Asn Lys Glu Gln
	1395 1400 1405
	Leu Thr Ala Thr Lys Thr Glu Gln Asn Ile Lys Asp Phe Glu Thr Ser
	1410 1415 1420
50	Asp Thr Phe Phe Gln Thr Ala Ser Gly Lys Asn Ile Ser Val Ala Lys
	1425 1430 1435 1440

5 Glu Leu Phe Asn Lys Ile Val Asn Phe Phe Asp Gln Lys Pro Glu Glu
 1445 1450 1455

 Leu His Asn Phe Ser Leu Asn Ser Glu Leu His Ser Asp Ile Arg Lys
 1460 1465 1470

10 Asn Lys Met Asp Ile Leu Ser Tyr Glu Glu Thr Asp Ile Val Lys His
 1475 1480 1485

15 Lys Ile Leu Lys Glu Ser Val Pro Val Gly Thr Gly Asn Gln Leu Val
 1490 1495 1500

20 Thr Phe Gln Gly Gln Pro Glu Arg Asp Glu Lys Ile Lys Glu Pro Thr
 1505 1510 1515 1520

25 Leu Leu Gly Phe His Thr Ala Ser Gly Lys Lys Val Lys Ile Ala Lys
 1525 1530 1535

30 Glu Ser Leu Asp Lys Val Lys Asn Leu Phe Asp Glu Lys Glu Gln Gly
 1540 1545 1550

 Thr Ser Glu Ile Thr Ser Phe Ser His Gln Trp Ala Lys Thr Leu Lys
 1555 1560 1565

35 Tyr Arg Glu Ala Cys Lys Asp Leu Glu Leu Ala Cys Glu Thr Ile Glu
 1570 1575 1580

40 Ile Thr Ala Ala Pro Lys Cys Lys Glu Met Gln Asn Ser Leu Asn Asn
 1585 1590 1595 1600

45 Asp Lys Asn Leu Val Ser Ile Glu Thr Val Val Pro Pro Lys Leu Leu
 1605 1610 1615

 Ser Asp Asn Leu Cys Arg Gln Thr Glu Asn Leu Lys Thr Ser Lys Ser
 1620 1625 1630

50 Ile Phe Leu Lys Val Lys Val His Glu Asn Val Glu Lys Glu Thr Ala
 1635 1640 1645

5 Lys Ser Pro Ala Thr Cys Tyr Thr Asn Gln Ser Pro Tyr Ser Val Ile
1650 1655 1660

10 Glu Asn Ser Ala Leu Ala Phe Tyr Thr Ser Cys Ser Arg Lys Thr Ser
1665 1670 1675 1680

15 Val Ser Gln Thr Ser Leu Leu Glu Ala Lys Lys Trp Leu Arg Glu Gly
1685 1690 1695

20 Ile Phe Asp Gly Gln Pro Glu Arg Ile Asn Thr Ala Asp Tyr Val Gly
1700 1705 1710

25 Asn Tyr Leu Tyr Glu Asn Asn Ser Asn Ser Thr Ile Ala Glu Asn Asp
1715 1720 1725

30 Lys Asn His Leu Ser Glu Lys Gln Asp Thr Tyr Leu Ser Asn Ser Ser
1730 1735 1740

35 Met Ser Asn Ser Tyr Ser Tyr His Ser Asp Glu Val Tyr Asn Asp Ser
1745 1750 1755 1760

40 Gly Tyr Leu Ser Lys Asn Lys Leu Asp Ser Gly Ile Glu Pro Val Leu
1765 1770 1775

45 Lys Asn Val Glu Asp Gln Lys Asn Thr Ser Phe Ser Lys Val Ile Ser
1780 1785 1790

50 Asn Val Lys Asp Ala Asn Ala Tyr Pro Gln Thr Val Asn Glu Asp Ile
1795 1800 1805

Cys Val Glu Glu Leu Val Thr Ser Ser Ser Pro Cys Lys Asn Lys Asn
1810 1815 1820

Ala Ala Ile Lys Leu Ser Ile Ser Asn Ser Asn Asn Phe Glu Val Gly
1825 1830 1835 1840

Pro Pro Ala Phe Arg Ile Ala Ser Gly Lys Ile Arg Leu Cys Ser His

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	1845	1850	1855
5	Glu Thr Ile Lys Lys Val Lys Asp Ile Phe Thr Asp Ser Phe Ser Lys 1860 1865 1870		
10	Val Ile Lys Glu Asn Asn Glu Asn Lys Ser Lys Ile Cys Gln Thr Lys 1875 1880 1885		
15	Ile Met Ala Gly Cys Tyr Glu Ala Leu Asp Asp Ser Glu Asp Ile Leu 1890 1895 1900		
20	His Asn Ser Leu Asp Asn Asp Glu Cys Ser Met His Ser His Lys Val 1905 1910 1915 1920		
25	Phe Ala Asp Ile Gln Ser Glu Glu Ile Leu Gln His Asn Gln Asn Met 1925 1930 1935		
30	Ser Gly Leu Glu Lys Val Ser Lys Ile Ser Pro Cys Asp Val Ser Leu 1940 1945 1950		
35	Glu Thr Ser Asp Ile Cys Lys Cys Ser Ile Gly Lys Leu His Lys Ser 1955 1960 1965		
40	Val Ser Ser Ala Asn Thr Cys Gly Ile Phe Ser Thr Ala Ser Gly Lys 1970 1975 1980		
45	Ser Val Gln Val Ser Asp Ala Ser Leu Gln Asn Ala Arg Gln Val Phe 1985 1990 1995 2000		
50	Ser Glu Ile Glu Asp Ser Thr Lys Gln Val Phe Ser Lys Val Leu Phe 2005 2010 2015		
	Lys Ser Asn Glu His Ser Asp Gln Leu Thr Arg Glu Glu Asn Thr Ala 2020 2025 2030		
	Ile Arg Thr Pro Glu His Leu Ile Ser Gln Lys Gly Phe Ser Tyr Asn 2035 2040 2045		

100

	Val Val Asn Ser Ser Ala Phe Ser Gly Phe Ser Thr Ala Ser Gly Lys
	2050 2055 2060
5	Gln Val Ser Ile Leu Glu Ser Ser Leu His Lys Val Lys Gly Val Leu
	2065 2070 2075 2080
10	Glu Glu Phe Asp Leu Ile Arg Thr Glu His Ser Leu His Tyr Ser Pro
	2085 2090 2095
15	Thr Ser Arg Gln Asn Val Ser Lys Ile Leu Pro Arg Val Asp Lys Arg
	2100 2105 2110
20	Asn Pro Glu His Cys Val Asn Ser Glu Met Glu Lys Thr Cys Ser Lys
	2115 2120 2125
25	Glu Phe Lys Leu Ser Asn Asn Leu Asn Val Glu Gly Gly Ser Ser Glu
	2130 2135 2140
30	Asn Asn His Ser Ile Lys Val Ser Pro Tyr Leu Ser Gln Phe Gln Gln
	2145 2150 2155 2160
35	Asp Lys Gln Gln Leu Val Leu Gly Thr Lys Val Ser Leu Val Glu Asn
	2165 2170 2175
40	Ile His Val Leu Gly Lys Glu Gln Ala Ser Pro Lys Asn Val Lys Met
	2180 2185 2190
45	Glu Ile Gly Lys Thr Glu Thr Phe Ser Asp Val Pro Val Lys Thr Asn
	2195 2200 2205
50	Ile Glu Val Cys Ser Thr Tyr Ser Lys Asp Ser Glu Asn Tyr Phe Glu
	2210 2215 2220
	Thr Glu Ala Val Glu Ile Ala Lys Ala Phe Met Glu Asp Asp Glu Leu
	2225 2230 2235 2240
	Thr Asp Ser Lys Leu Pro Ser His Ala Thr His Ser Leu Phe Thr Cys
	2245 2250 2255
	Pro Glu Asn Glu Glu Met Val Leu Ser Asn Ser Arg Ile Gly Lys Arg

101

	2260	2265	2270
5	Arg Gly Glu Pro Leu Ile Leu Val	Gly Glu Pro Ser Ile Lys Arg Asn	
	2275	2280	2285
10	Leu Leu Asn Glu Phe Asp Arg Ile Ile Glu Asn Gln Glu Lys Ser Leu		
	2290	2295	2300
15	Lys Ala Ser Lys Ser Thr Pro Asp Gly Thr Ile Lys Asp Arg Arg Leu		
	2305	2310	2315 2320
20	Phe Met His His Val Ser Leu Glu Pro Ile Thr Cys Val Pro Phe Arg		
	2325	2330	2335
25	Thr Thr Lys Glu Arg Gln Glu Ile Gln Asn Pro Asn Phe Thr Ala Pro		
	2340	2345	2350
30	Gly Gln Glu Phe Leu Ser Lys Ser His Leu Tyr Glu His Leu Thr Leu		
	2355	2360	2365
35	Glu Lys Ser Ser Ser Asn Leu Ala Val Ser Gly His Pro Phe Tyr Gln		
	2370	2375	2380
40	Val Ser Ala Thr Arg Asn Glu Lys Met Arg His Leu Ile Thr Thr Gly		
	2385	2390	2395 2400
45	Arg Pro Thr Lys Val Phe Val Pro Pro Phe Lys Thr Lys Ser His Phe		
	2405	2410	2415
50	His Arg Val Glu Gln Cys Val Arg Asn Ile Asn Leu Glu Glu Asn Arg		
	2420	2425	2430
	Gln Lys Gln Asn Ile Asp Gly His Gly Ser Asp Asp Ser Lys Asn Lys		
	2435	2440	2445
	Ile Asn Asp Asn Glu Ile His Gln Phe Asn Lys Asn Asn Ser Asn Gln		
	2450	2455	2460
	Ala Ala Ala Val Thr Phe Thr Lys Cys Glu Glu Glu Pro Leu Asp Leu		
	2465	2470	2475 2480

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	Ile Thr Ser Leu Gln Asn Ala Arg Asp Ile Gln Asp Met Arg Ile Lys
	2485 2490 2495
5	Lys Lys Gln Arg Gln Arg Val Phe Pro Gln Pro Gly Ser Leu Tyr Leu
	2500 2505 2510
10	Ala Lys Thr Ser Thr Leu Pro Arg Ile Ser Leu Lys Ala Ala Val Gly
	2515 2520 2525
15	Gly Gln Val Pro Ser Ala Cys Ser His Lys Gln Leu Tyr Thr Tyr Gly
	2530 2535 2540
20	Val Ser Lys His Cys Ile Lys Ile Asn Ser Lys Asn Ala Glu Ser Phe
	2545 2550 2555 2560
	Gln Phe His Thr Glu Asp Tyr Phe Gly Lys Glu Ser Leu Trp Thr Gly
	2565 2570 2575
25	Lys Gly Ile Gln Leu Ala Asp Gly Gly Trp Leu Ile Pro Ser Asn Asp
	2580 2585 2590
30	Gly Lys Ala Gly Lys Glu Glu Phe Tyr Arg Ala Leu Cys Asp Thr Pro
	2595 2600 2605
35	Gly Val Asp Pro Lys Leu Ile Ser Arg Ile Trp Val Tyr Asn His Tyr
	2610 2615 2620
40	Arg Trp Ile Ile Trp Lys Leu Ala Ala Met Glu Cys Ala Phe Pro Lys
	2625 2630 2635 2640
45	Glu Phe Ala Asn Arg Cys Leu Ser Pro Glu Arg Val Leu Leu Gln Leu
	2645 2650 2655
	Lys Tyr Arg Tyr Asp Thr Glu Ile Asp Arg Ser Arg Arg Ser Ala Ile
	2660 2665 2670
50	Lys Lys Ile Met Glu Arg Asp Asp Thr Ala Ala Lys Thr Leu Val Leu
	2675 2680 2685

	Cys Val Ser Asp Ile Ile Ser Leu Ser Ala Asn Ile Ser Glu Thr Ser
	2690 2695 2700
5	Ser Asn Lys Thr Ser Ser Ala Asp Thr Gln Lys Val Ala Ile Ile Glu
	2705 2710 2715 2720
10	Leu Thr Asp Gly Trp Tyr Ala Val Lys Ala Gln Leu Asp Pro Pro Leu
	2725 2730 2735
15	Leu Ala Val Leu Lys Asn Gly Arg Leu Thr Val Gly Gln Lys Ile Ile
	2740 2745 2750
20	Leu His Gly Ala Glu Leu Val Gly Ser Pro Asp Ala Cys Thr Pro Leu
	2755 2760 2765
	Glu Ala Pro Glu Ser Leu Met Leu Lys Ile Ser Ala Asn Ser Thr Arg
	2770 2775 2780
25	Pro Ala Arg Trp Tyr Thr Lys Leu Gly Phe Phe Pro Asp Pro Arg Pro
	2785 2790 2795 2800
30	Phe Pro Leu Pro Leu Ser Ser Leu Phe Ser Asp Gly Gly Asn Val Gly
	2805 2810 2815
35	Cys Val Asp Val Ile Ile Gln Arg Ala Tyr Pro Ile Gln Arg Met Glu
	2820 2825 2830
40	Lys Thr Ser Ser Gly Leu Tyr Ile Phe Arg Asn Glu Arg Glu Glu Glu
	2835 2840 2845
	Lys Glu Ala Ala Lys Tyr Val Glu Ala Gln Gln Lys Arg Leu Glu Ala
	2850 2855 2860
45	Leu Phe Thr Lys Ile Gln Glu Glu Phe Glu Glu His Glu Glu Asn Thr
	2865 2870 2875 2880
50	Thr Lys Pro Tyr Leu Pro Ser Arg Ala Leu Thr Arg Gln Gln Val Arg
	2885 2890 2895

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Ala Leu Gln Asp Gly Ala Glu Leu Tyr Glu Ala Val Lys Asn Ala Ala
2900 2905 2910

5

Asp Pro Ala Tyr Leu Glu Gly Tyr Phe Ser Glu Glu Gln Leu Arg Ala
2915 2920 2925

10

Leu Asn Asn His Arg Gln Met Leu Asn Asp Lys Lys Gln Ala Gln Ile
2930 2935 2940

15

Gln Leu Glu Ile Arg Lys Ala Met Glu Ser Ala Glu Gln Lys Glu Gln
2945 2950 2955 2960

20

Gly Leu Ser Arg Asp Val Thr Thr Val Trp Lys Leu Arg Ile Val Ser
2965 2970 2975

25

Tyr Ser Lys Lys Glu Lys Asp Ser Val Ile Leu Ser Ile Trp Arg Pro
2980 2985 2990

30

Ser Ser Asp Leu Tyr Ser Leu Leu Thr Glu Gly Lys Arg Tyr Arg Ile
2995 3000 3005

35

Tyr His Leu Ala Thr Ser Lys Ser Lys Ser Lys Ser Glu Arg Ala Asn
3010 3015 3020

Ile Gln Leu Ala Ala Thr Lys Lys Thr Gln Tyr Gln Gln Leu Pro Val
3025 3030 3035 3040

40

Ser Asp Glu Ile Leu Phe Gln Ile Tyr Gln Pro Arg Glu Pro Leu His
3045 3050 3055

45

Phe Ser Lys Phe Leu Asp Pro Asp Phe Gln Pro Ser Cys Ser Glu Val
3060 3065 3070

50

Asp Leu Ile Gly Phe Val Val Ser Val Val Lys Lys Thr Gly Leu Ala
3075 3080 3085

Pro Phe Val Tyr Leu Ser Asp Glu Cys Tyr Asn Leu Leu Ala Ile Lys
3090 3095 3100

5 Phe Trp Ile Asp Leu Asn Glu Asp Ile Ile Lys Pro His Met Leu Ile
3105 3110 3115 3120

Ala Ala Ser Asn Leu Gln Trp Arg Pro Glu Ser Lys Ser Gly Leu Leu
3125 3130 3135

10 Thr Leu Phe Ala Gly Asp Phe Ser Val Phe Ser Ala Ser Pro Lys Glu
3140 3145 3150

15 Gly His Phe Gln Glu Thr Phe Asn Lys Met Lys Asn Thr Val Glu Asn
3155 3160 3165

20 Ile Asp Ile Leu Cys Asn Glu Ala Glu Asn Lys Leu Met His Ile Leu
3170 3175 3180

25 His Ala Asn Asp Pro Lys Trp Ser Thr Pro Thr Lys Asp Cys Thr Ser
3185 3190 3195 3200

Gly Pro Tyr Thr Ala Gln Ile Ile Pro Gly Thr Gly Asn Lys Leu Leu
3205 3210 3215

30 Met Ser Ser Pro Asn Cys Glu Ile Tyr Tyr Gln Ser Pro Leu Ser Leu
3220 3225 3230

35 Cys Met Ala Lys Arg Lys Ser Val Ser Thr Pro Val Ser Ala Gln Met
3235 3240 3245

40 Thr Ser Lys Ser Cys Lys Gly Glu Lys Glu Ile Asp Asp Gln Lys Asn
3250 3255 3260

45 Cys Lys Lys Arg Arg Ala Leu Asp Phe Leu Ser Arg Leu Pro Leu Pro
3265 3270 3275 3280

50 Pro Pro Val Ser Pro Ile Cys Thr Phe Val Ser Pro Ala Ala Gln Lys
3285 3290 3295

Ala Phe Gln Pro Pro Arg Ser Cys Gly Thr Lys Tyr Glu Thr Pro Ile
3300 3305 3310

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5 Lys Lys Lys Glu Leu Asn Ser Pro Gln Met Thr Pro Phe Lys Lys Phe
3315 3320 3325

Asn Glu Ile Ser Leu Leu Glu Ser Asn Ser Ile Ala Asp Glu Glu Leu
3330 3335 3340

10 Ala Leu Ile Asn Thr Gln Ala Leu Leu Ser Gly Ser Thr Gly Glu Lys
3345 3350 3355 3360

15 Gln Phe Ile Ser Val Ser Glu Ser Thr Arg Thr Ala Pro Thr Ser Ser
3365 3370 3375

20 Glu Asp Tyr Leu Arg Leu Lys Arg Arg Cys Thr Thr Ser Leu Ile Lys
3380 3385 3390

25 Glu Gln Glu Ser Ser Gln Ala Ser Thr Glu Glu Cys Glu Lys Asn Lys
3395 3400 3405

Gln Asp Thr Ile Thr Thr Lys Lys Tyr Ile
3410 3415

30

- (2) INFORMATION FOR SEQ ID NO:5:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 19
- (B) TYPE: amino acid
- 35 (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: no
- (iv) ANTI-SENSE: no
- 40 (v) ORIGINAL SOURCE
- (A) ORGANISM: Homo sapiens sapiens
- (C) INDIVIDUAL/ISOLATE:
- (D) DEVELOPMENTAL STAGE: adult
- (F) TISSUE TYPE: female breast
- 45 (G) CELL TYPE: normal breast tissue
- (H) CELL LINE: HMEC

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(I) ORGANELLE: no

(ix) FEATURE:

(A) NAME/KEY: BRCA1 C-19 antigen

(B) LOCATION: 1845 to 1863

5 (C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

10 (C) JOURNAL:

(D) VOLUME:

(E) PAGES:

(F) DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:5

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Tyr Gln Cys Gln Glu Leu Asp Thr Tyr Leu Ile Pro Gln Ile Pro His

1 5 10 15

20 Ser His Tyr

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

30 (iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) ORIGINAL SOURCE

(A) ORGANISM: Homo sapiens sapiens

(C) INDIVIDUAL/ISOLATE:

35 (D) DEVELOPMENTAL STAGE: adult

(F) TISSUE TYPE: female breast

(G) CELL TYPE: normal breast tissue

(H) CELL LINE: HMEC

108

- (I) ORGANELLE: no
- (ix) FEATURE:
- (A) NAME/KEY: BRCA1 C-20 antigen
- (B) LOCATION: 1844 to 1863
- 5 (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION:
- (x) PUBLICATION INFORMATION:
- (A) AUTHORS:
- (B) TITLE:
- 10 (C) JOURNAL:
- (D) VOLUME:
- (E) PAGES:
- (F) DATE:
- (K) RELEVANT RESIDUES IN SEQ ID NO:6
- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
- Leu Tyr Gln Cys Gln Glu Leu Asp Thr Tyr Leu Ile Pro Gln Ile Pro
- 1 5 10 15
- 20 His Ser His Tyr
- 20
- 25 (2) INFORMATION FOR SEQ ID NO:7:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- 30 (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: no
- (iv) ANTI-SENSE: no
- (v) ORIGINAL SOURCE
- 35 (A) ORGANISM: Homo sapiens sapiens
- (C) INDIVIDUAL/ISOLATE:
- (D) DEVELOPMENTAL STAGE: adult
- (F) TISSUE TYPE: female breast

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(G) CELL TYPE: normal breast tissue

(H) CELL LINE: HMEC

(I) ORGANELLE: no

(ix) FEATURE:

5 (A) NAME/KEY: BRCA1 D-20 antigen

(B) LOCATION: 1 to 20

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

10 (A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) PAGES:

15 (F) DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:7

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

20 Met Asp Leu Ser Ala Leu Arg Val Glu Glu Val Gln Asn Val Ile Asn
1 5 10 15Ala Met Gln Lys
20

25

5 Thus, although there have been described particular embodiments of the present invention of a new and useful Characterized BRCA1 and BRCA2 Proteins and Screening and Therapeutic Methods Based on Characterized BRCA1 and BRCA2 Proteins, it is not intended that such references be construed as limitations upon the scope of this invention except as set forth in the following claims. Further, although there have been described certain examples used in the preferred embodiment, it is not intended that such examples be construed as limitations upon the scope of this invention except as set forth in the following claims.

CLAIMS

What is claimed is:

1. A method for isolating a receptor for the BRCA1 protein, the method comprising the steps of:
 - 5 (a) contacting cells or cell lysates having the BRCA1 receptor with BRCA1; and
 - (b) isolating the receptor which binds with BRCA1.
2. The method according to claim 1 wherein the cells having the BRCA1 receptor are identified by the steps of:
 - 10 (a) labelling the BRCA1;
 - (b) screening cell cultures with the labelled BRCA1; and
 - (c) isolating cells that bind an elevated amount of the labelled BRCA1.
3. The method according to claim 2 wherein the BRCA1 receptor is isolated by lysing the cells and passing the cell lysate over a column containing the BRCA1 bound to a solid phase matrix within the column.
4. The method according to claim 2 wherein the BRCA1 receptor is isolated by constructing a cDNA library from the cells binding the BRCA1 receptor; transfecting the cDNA library into a cell line that does not exhibit binding of the BRCA1 receptor; screening the cell line for newly acquired specific binding; isolating DNA from cells exhibiting specific binding; and sequencing the isolated DNA to determine the DNA sequence for the BRCA1 receptor.
5. The method according to claim 2 wherein the BRCA1 is labelled by binding the BRCA1 to an immunoglobulin.
6. The method according to claim 5 wherein the BRCA1 receptor is isolated by immunoprecipitation of the BRCA1 receptor-BRCA1-immunoglobulin complex.
7. The method according to claim 5 wherein the BRCA1 receptor is isolated using flow cytometry.
8. A method of treating breast or ovarian cancer in a patient, the method comprising the step of administering a therapeutically effective amount of a BRCA1 targeted growth inhibitor agent so that the agent contacts a receptor on the surface of breast or ovarian cancer cells in the patient.
9. A method of treating breast or ovarian cancer in a patient, the method comprising the steps of:

(a) ligating a gene that encodes the BRCA1 receptor with a promoter capable of inducing expression of the gene in a breast or ovarian cancer cell;

(b) introducing the ligated gene into a breast or ovarian cancer cell in the patient; and

(c) administering a therapeutically effective amount of a targeted growth inhibitor agent so that the agent contacts a BRCA1 receptor on a surface of the breast or ovarian cancer cells in the patient.

10. A method of treating breast or ovarian cancer in a patient, the method comprising the steps of:

(a) isolating a gene that encodes the BRCA1 receptor;

(b) ligating the gene that encodes the BRCA1 receptor with a promoter capable of inducing expression of the gene in a breast or ovarian cancer cell;

(c) introducing the ligated gene into a breast or ovarian cancer cell in the patient; and

(d) administering a therapeutically effective amount of a targeted growth inhibitor agent so that the agent contacts a BRCA1 receptor on a surface of the breast or ovarian cancer cells in the patient.

11. A method for identifying compounds which mimic a peptide structure of a BRCA1 protein comprising a carboxy terminal sequence substantially identical to the carboxy terminal sequence of an amino acid sequence as essentially set forth in SEQ ID NO:2 and having the following characteristic: molecular weight of substantially 190 kDa as determined by non-reduced sodium dodecylsulfate polyacrylamide gel electrophoresis, the method comprising the steps of:

a. determining domains of the protein that are essential for growth inhibitor activity;

b. analyzing structure and function of the domains of the protein that are essential for growth inhibitor activity;

c. comparing the structure and function of the domains of the protein that are essential for growth inhibitor activity to other compounds; and

d. determining which compounds have structure so as to mimic the structure and function of the agent.

12. A method of treating ovarian cancer in a patient comprising the steps of ligating a gene that encodes a protein having an amino acid sequence

as essentially set forth in SEQ ID NO:2 with a promoter capable of inducing expression of the gene in a ovarian cancer cell and introducing the ligated gene into a ovarian cancer cell.

5 13. The method of treating ovarian cancer described in claim 12 wherein the gene has a DNA sequence selected from among:

- (a) the DNA sequence as essentially set forth in SEQ ID NO:1 or its complementary strands;
- (b) a DNA sequence which hybridizes to SEQ ID NO:1 or fragments thereof; and
- 10 (c) DNA sequences which but for the degeneracy of the genetic code would hybridize to the DNA sequences defined in (a) and (b).

15 14. The method of treating ovarian cancer described in claim 12 wherein the gene has a DNA sequence having 20-99% homology with SEQ ID NO:1.

15 15. The method according to claim 12 wherein the ligated gene is introduced into the cell in a viral expression vector.

16 16. The method according to claim 12 wherein the ovarian cancer is gene-linked hereditary ovarian cancer.

20 17. The method described in claim 12 wherein the ovarian cancer is sporadic ovarian cancer.

25 18. A method of treating breast cancer in a patient comprising the steps of ligating a gene that encodes a protein having an amino acid sequence as essentially set forth in SEQ ID NO:4 with a promoter capable of inducing expression of the gene in a breast cancer cell and introducing the ligated gene into a breast cancer cell.

30 19. The method of treating breast cancer described in claim 18 wherein the gene has a DNA sequence selected from among:

- (a) the DNA sequence as essentially set forth in SEQ ID NO:3 or its complementary strands;
- (b) a DNA sequence which hybridizes to SEQ ID NO:3 or fragments thereof; and
- (c) DNA sequences which but for the degeneracy of the genetic code would hybridize to the DNA sequences defined in (a) and (b).

35 20. The method of treating breast cancer described in claim 18 wherein the gene has a DNA sequence having 20-99% homology with SEQ ID

NO:3.

21. The method according to claim 18 wherein the ligated gene is introduced into the cell in a viral expression vector.

22. The method according to claim 18 wherein the breast cancer is gene-linked hereditary breast cancer.

23. The method described in claim 18 wherein the breast cancer is sporadic breast cancer.

24. A method of treating ovarian cancer in a patient comprising the steps of ligating a gene that encodes a protein having an amino acid sequence as essentially set forth in SEQ ID NO:4 with a promoter capable of inducing expression of the gene in a ovarian cancer cell and introducing the ligated gene into a ovarian cancer cell.

25. The method of treating ovarian cancer described in claim 24 wherein the gene has a DNA sequence selected from among:

- (a) the DNA sequence as essentially set forth in SEQ ID NO:3 or its complementary strands;
- (b) a DNA sequence which hybridizes to SEQ ID NO:3 or fragments thereof; and
- (c) DNA sequences which but for the degeneracy of the genetic code would hybridize to the DNA sequences defined in (a) and (b).

26. The method of treating ovarian cancer described in claim 24 wherein the gene has a DNA sequence having 20-99% homology with SEQ ID NO:3.

27. The method according to claim 24 wherein the ligated gene is introduced into the cell in a viral expression vector.

28. The method according to claim 24 wherein the ovarian cancer is gene-linked hereditary ovarian cancer.

29. The method described in claim 24 wherein the ovarian cancer is sporadic ovarian cancer.

30. A method of treating breast or ovarian cancer comprising the steps of:

- (a) incubating a liposome preparation with a DNA segment that encodes the protein as essentially set forth in SEQ ID NO:2 or with a DNA segment that encodes the protein as essentially set forth in SEQ ID NO:4;

- (b) transfecting a breast or ovarian cancer cell with the DNA liposome complex of step (a).

31. The method according to claim 30 wherein the liposome preparation is a cationic liposome preparation.

5 32. A method of treating breast or ovarian cancer comprising the steps of:

- (a) delivering anti-sense BRCA1 DNA or anti-sense BRCA2 DNA to breast or ovarian cancer cells within a patient; and

(b) administering a therapeutically effective amount of a chemotherapeutic drug to the patient.

10 33. A method for isolating a cellular receptor for the BRCA2 protein, the method comprising the steps of:

(a) contacting cells and cell lysates having the BRCA2 receptor with a protein having an amino acid sequence as essentially set forth in SEQ ID NO:4; and

- (b) isolating the receptor that binds the protein.

15 34. The method according to claim 33 wherein cells having the BRCA2 receptor are identified by the steps of:

(a) labelling the protein as essentially set forth in SEQ ID NO:4;

20 (b) screening cell cultures with the labelled protein; and

(d) isolating cells that bind an elevated amount of the labelled protein.

25 35. The method according to claim 34 wherein the BRCA2 receptor is isolated by lysing the cells and isolating the BRCA2 receptor by passing the cell lysate over a column containing the protein as essentially set forth in SEQ ID NO:4 bound to a solid phase matrix within the column.

30 36. The method according to claim 34 wherein the BRCA2 receptor is isolated by constructing a cDNA library from the cells expressing high levels of BRCA2 receptor; transfecting the cDNA library into a cell line that does not exhibit binding of the protein as essentially set forth in SEQ ID NO:4 to a receptor; screening the cell line for newly acquired specific binding; isolating DNA from cells exhibiting specific binding; and sequencing the isolated DNA to determine the DNA sequence for the BRCA2 receptor.

35 37. The method according to claim 34 wherein the protein is labelled by binding the protein to a immunoglobulin.

38. The method according to claim 37 wherein the BRCA2 receptor is isolated by immunoprecipitation of the BRCA2 receptor-protein-

immunoglobulin complex.

39. The method according to claim 37 wherein the BRCA2 receptor is isolated using flow cytometry.

5 40. A cleavage product of BRCA1 wherein the cleavage product comprises a carboxy terminal sequence substantially identical to the carboxy terminal sequence of a protein having an amino acid sequence as essentially set forth in SEQ ID NO:2 and has the following characteristic: molecular weight of substantially 70 kDa as determined by non-reduced sodium dodecylsulfate polyacrylamide gel electrophoresis.

10 41. The cleavage product according to claim 40 having the following additional characteristics:

(a) cross-reacts with antisera against the protein having an amino acid sequence as essentially set forth in SEQ ID NO:2, and

15 (b) addition of a peptide derived from the carboxy terminal sequence of the protein having an amino acid sequence as essentially set forth in SEQ ID NO:2 blocks cross-reaction with antisera against the protein having an amino acid sequence as essentially set forth in SEQ ID NO:2.

42. The cleavage product according to claim 40 where the cleavage product has a amino acid sequence that includes a granin box domain.

20 43. The cleavage product according to claim 40 wherein the cleavage product has the following additional characteristic: is localized in the nuclear fraction of breast epithelial cells.

44. An expression vector comprising a DNA segment encoding the cleavage product in claim 40.

25 45. A process for the production of a recombinant host cell comprising inserting therein the expression vector according to claim 45.

46. A recombinant host cell produced by the process of claim 45.

30 47. A process for producing a BRCA1 cleavage product which comprises culturing a recombinant host cell, said recombinant host cell including the expression vector described in claim 44, in a suitable nutrient medium until the targeted growth inhibitor agent is formed and thereafter isolating the agent.

48. The method of claim 8, wherein the breast or ovarian cancer is sporadic breast or ovarian cancer.

35 49. The method of claim 8, wherein the BRCA1 targeted growth inhibitor agent is BRCA1 as essentially set forth in SEQ ID NO: 2.

50. A purified and isolated receptor which occurs on the surface of breast or ovarian epithelial cells and which is bound by BRCA1.

51. A method of screening a compound for tumor suppressor activity comprising contacting the compounds with the receptor of claim 50, a compound which binds the receptor indicating a compound having potential tumor suppressor activity.

52. The method of claim 51, wherein the compound is a BRCA1 cleavage fragment.

53. The method of claim 51, wherein the receptor is expressed on the surface of a cell.

54. A purified and isolated receptor which occurs on the surface of breast or ovarian epithelial cells and which is bound by BRCA2.

55. A method of screening a compound for tumor suppressor activity comprising contacting the compounds with the receptor of claim 54, a compound which binds the receptor indicating a compound having potential tumor suppressor activity.

56. The method of claim 55, wherein the compound is a BRCA2 cleavage product.

57. The method of claim 55, wherein the receptor is expressed on the surface of a cell.

58. A protein having tumor suppressor activity and comprising a granin box consensus sequence shown in figure 5 wherein the protein is not the BRCA1 or BRCA2.

59. The protein of claim 58, wherein the tumor suppressor activity is specific for breast and ovarian cancer.

60. A method of preventing sporadic breast or ovarian cancer in a patient, the method comprising administering a prophylactically effective amount of a BRCA1 or BRCA2 targeted growth inhibitor agent so that the agent contacts a receptor on the surface of breast or ovarian cancer cells in the patient and prevents sporadic breast or ovarian cancer.

61. The method of claim 60, wherein the cancer is prevented by administering a BRCA1 targeted growth inhibitor agent.

62. The method of claim 61, wherein the BRCA1 targeted growth inhibitor agent is BRCA1 as essentially set forth in SEQ ID NO: 2.

63. The method of claim 60, wherein the cancer is prevented by administering a BRCA2 targeted growth inhibitor agent.

64. The method of claim 63, wherein the BRCA2 targeted growth inhibitor agent is BRCA2 as essentially set forth in SEQ ID NO: 4.

5 65. A method of treating breast or ovarian cancer in patient, the method comprising the step of administering a therapeutically effective amount of a BRCA2 targeted growth inhibitor agent so that the agent contacts a receptor on the surface of breast or ovarian cancer cells in the patient.

66. The method of claim 65, wherein the breast or ovarian cancer is sporadic breast or ovarian cancer.

10 67. The method of claim 65, wherein the BRCA2 targeted growth inhibitor agent is BRCA2 as essentially set forth in SEQ ID NO: 4.

15 68. A method of treating breast or ovarian cancer in a patient, the method comprising the step of administering a therapeutically effective amount of a compound which binds the receptor for either BRCA1 or BRCA2 and acts as an agonist of the tumor suppressor activity.

Figure 1

Figure 1: BRCA1 Antigens

C-19 (19 C-terminal amino acids): [Seq ID No: 5]

Tyr Gln Cys Gln Glu Leu Asp Thr Tyr Leu Ile Pro Gln Ile Pro
His Ser His Tyr

C-20 (20 C-terminal amino acids): [Seq ID No: 6]

Leu Tyr Gln Cys Gln Glu Leu Asp Thr Tyr Leu Ile Pro Gln Ile
Pro His Ser His Tyr

D-20 (20 N-terminal amino acids): [Seq ID No: 7]

Met Asp Leu Ser Ala Leu Arg Val Glu Glu Val Gln Asn Val Ile
Asn Ala Met Gln Lys

Figure 6

Table I Effect of BRCA1 Expression Vectors on Growth

Vector	Fibroblast	MCF-7	CaOV-4	Lung Ca	Colon Ca
LXSN	85+2.5	85+3.7	72+2.3	98_1.7	433+9.4
BRCA1	87+2.2	0+0*	0+0*	101+4.2	480+16.3
Δ343-1081	84+1.4	96+3.7	76+4.9	97+3.7	460+29.4
Δ515-1092	88+2.4	93+15.9	77+4.2	99+5.0	473+28.7
1835 Stop	85+1.2	88+3.3	3+1.7	102+5.8	473+20.5
340 Stop	87+1.4	89+3.3	80+2.7	99+5.0	483+33.0

G418-resistant transfectants per 107 cells, Mean + Standard Error

Lung cancer cells = FK111; colon cancer cells = OK3;

Breast cancer cell line = MCF-7; Ovarian cancer cell line = CaOV-4

* 10-20 small colonies were identified in each transfection but these
never grew beyond 30 cells per clone.

Figure 2

Table of the Genetic Code

Amino Acids			Codons							
Alanine	Ala	A	GCA	GCC	GCG	GCU				
Cysteine	Cys	C	UGC	UGU						
Aspartic acid	Asp	D	GAC	CAU						
Glutamic acid	Glu	E	GAA	GAG						
Phenylalanine	Phe	F	UUC	UUU						
Glycine	Gly	G	GGA	GGC	GGG	GGU				
Histidine	His	H	CAC	CAU						
Isoleucine	Ile	I	AUA	AUC	AUU					
Lysine	Lys	K	AAA	AAG						
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU		
Methionine	Met	M	AUG							
Asparagine	Asn	N	AAC	AAU						
Proline	Pro	P	CCA	CCC	CCG	CCU				
Glutamine	Gln	Q	CAA	CAG						
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU		
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU		
Threonine	Thr	T	ACA	ACC	ACG	ACU				
Valine	Val	V	GUA	GUC	GUG	GUU				
Tryptophan	Trp	W	UGG							
Tyrosine	Tyr	Y	UAC	UAU						

Figure 3

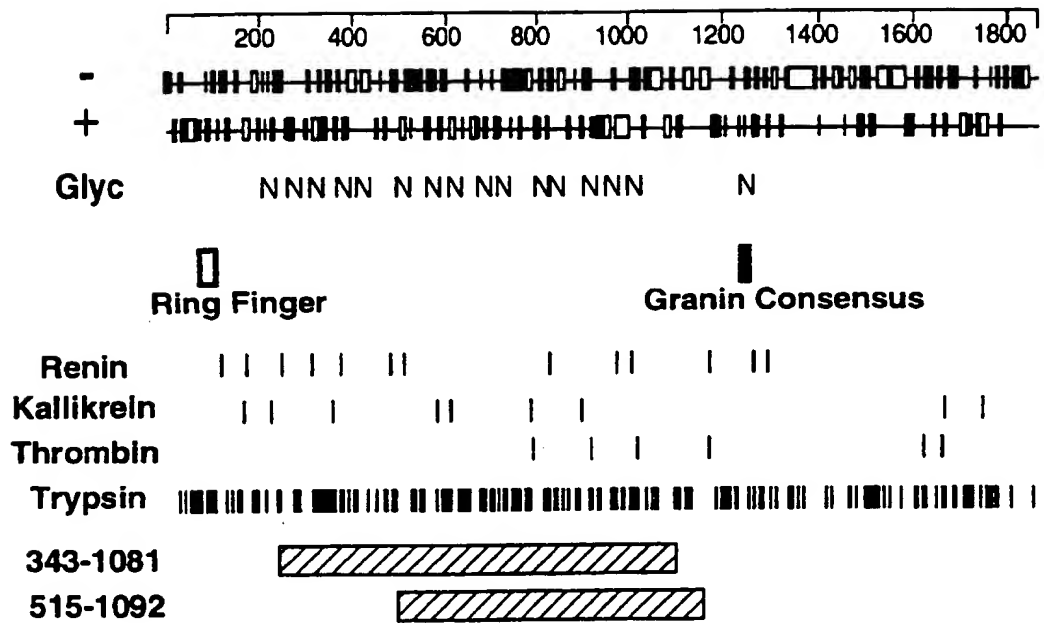
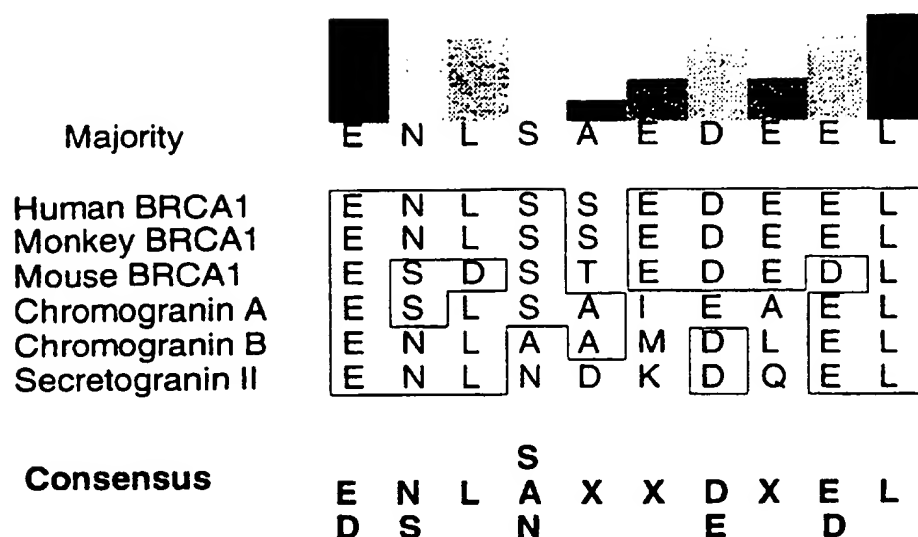


Fig 4



The probability that BRCA1 would contain a polypeptide that would satisfy the granin consensus by chance alone is approximately 1 in 55. This calculation is based on the following rationale:

$$(N-n+1) \prod_{i=1}^n \sum_{k=1}^k A_i$$

Where n = length of the consensus sequence
 k = number of alternative amino acids at site i of the consensus
 A_i = frequency of amino acid i in the entire sequence N amino acids long

AA1	AA2	AA3	AA4	AA5	AA6	AA7	AA8	AA9	AA10	N-n+1	Probability
E	N	L	A	X	X	D	X	E	L		
D	L		N			E		D			
0.15	0.19	0.08	0.23	1.0	1.0	0.15	1.0	0.15	0.08	1854	= 0.0018

Note that this does not take into account the likelihood of amino acid pairs that frequently co-occur.

Figure 5

Granin Sequences		Amino Acid									
Granin	Species	E	N	L	S	X	X	D	X	E	L
Consensus		D	S		A			E		D	
					N						
BRCA1	Human	E	N	L	S	S	E	D	E	E	L
	Rhesus	E	N	L	S	S	E	D	E	E	L
	Mouse	E	S	D	S	T	E	D	E	D	L
BRCA2	Human	E	S	N	S	I	A	D	E	E	L
Chromogranin A	Human	E	S	L	S	A	I	E	A	E	L
	Bovine	E	S	L	S	A	I	E	A	E	L
	Rat	E	S	L	S	A	I	E	A	E	L
	Pig	E	S	L	S	A	I	E	A	E	L
Chromogranin B	Human	E	N	L	A	A	M	D	L	E	L
	Bovine	E	N	L	A	A	M	D	L	E	L
	Mouse	E	N	L	A	A	M	D	L	E	L
Secretogranin II	Human	E	N	L	N	D	K	D	Q	E	L
	Bovine	E	N	L	N	D	K	D	Q	E	L
	Rat	D	N	L	N	D	K	D	Q	E	L
	Mouse	E	N	L	N	-	-	D	Q	E	L
Secretogranin III	Rat	E	N	L	D	E	T	I	A	L	Q
	Mouse	E	N	L	D	E	T	I	A	L	Q
Secretogranin V	Human	G	N	I	P	N	I	V	A	E	L
	Pig	G	N	I	P	N	I	V	A	E	L
	Rat	G	N	I	P	N	I	V	A	E	L
	Xenopus	G	N	I	P	N	I	V	A	E	L

Frequency of consensus amino acid in complete BRCA1 sequence 0.15 0.19 0.08 0.23 1 1 0.15 1 0.15 0.08

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Figure 7

Table II. Inherited BRCA1 mutation and type of cancer

Termination codon of mutant protein	Cancer site	
	Breast	Ovary
0a	16	3
36	2	
37	7	1
39	17	9
64	6	4
81	4	2
313	5	1
766	3	4
780	7	
901	14	4
915	4	3
123	6	
1214-1223	Grannin motif	
1265	5	
1364	12	1
1829	6	
1853	7	
1863b	13	
0-1223	91	31 25%
1223-1863	43	1 2%

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Figure 8

Table III. Inhibition of Tumorigenesis by BRCA1

Vector	MCF-7 (4wks)	MCF-7 (8wks)	Weight of MCF Tumor	MCF-7 stables	Established tumors	Colon Tumors*
None	6/6	6/6*	Not done	Not done	Not done	5/6
BRCA1	0/6	4/6*	60g+24	0/20	24.4+2.1#	6/6
Δ343-1081	5/6	6/6*	569g+60	13/15	8.6+1.3#	6/6

The columns headed MCF-7 (4wks) and (8wks) and colon tumors are results following retroviral transduction of cultured cells. The assay for inhibition of established tumor growth was whether the retrovirus could delay survival for an additional 14 days. The column labeled MCF-7 stables shows tumor development of cloned BRCA1 and mutant cell lines. MCF-7 stables are results of stable transformants.

*colon tumor weights: BRCA1=1540+128; Δ343-1081=1633+110

#mean+SE of post injection survivals (days): BRCA1=15,18,22,26,41
Δ343-1081=4,8,9,11,11

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Fig. 9

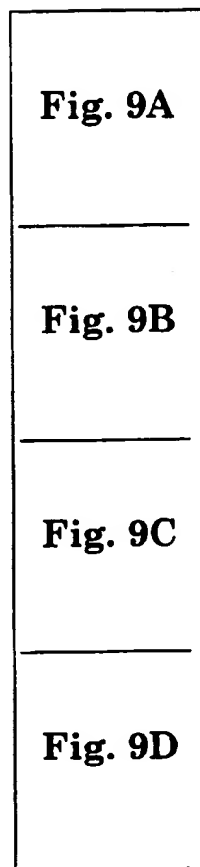


Fig. 9A**Gene sequence for BRCA1 [SEQ. ID. NO.1] (reference Miki et. al. Science 266:66.1994.)**

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ggaggccttcaccctctgctctgggtaaagttcattggaacagaaagaaatggatttatctgctcttcgcgt
tgaagaagtacaaaatgtcattaatgctatgcagaaaatcttagagtgtcccatctgtctggagttgatcaa
ggaacctgtctccacaaagtgtagccacatatgttgcgaatttgcagtgctgaaacttctcaaccagaagaa
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gtcaacttgttgaagagctatgaaaaatcttctgtctttcagcttgacacagggttggagtatgcaaacag
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Fig. 9B

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Fig. 9C

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Fig. 9D

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Fig. 10

Fig. 10A
Fig. 10B
Fig. 10C
Fig. 10D
Fig. 10E
Fig. 10F

Fig. 10A**Sequence of the BRCA2 cDNA [SEQ ID NO:3]**

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Fig. 10B

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Fig. 10C

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Fig. 10D

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Fig. 10E

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Fig. 10F

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Fig. 11

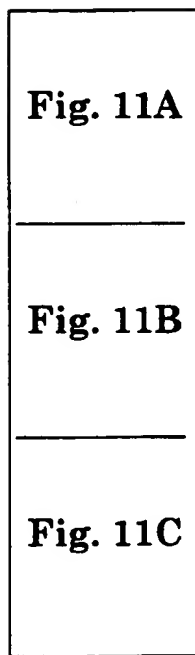


Fig. 11A**BRCA2 protein sequence [SEQ ID NO:4]**

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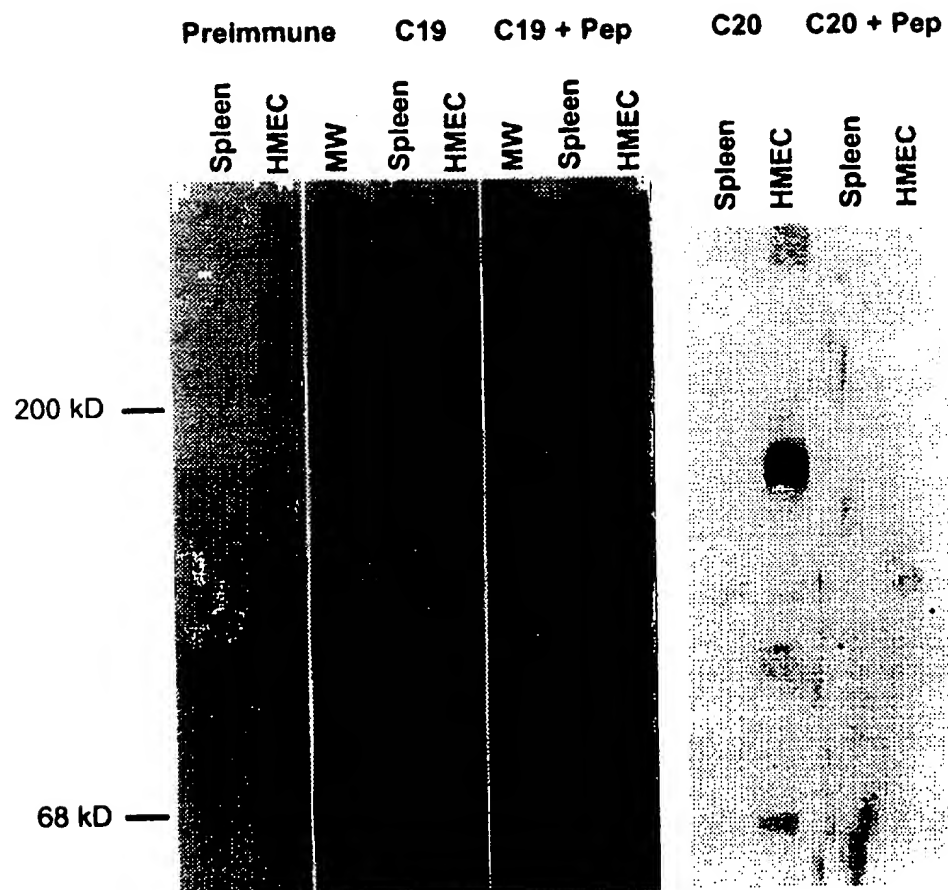
Fig. 11B

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Fig. 11C

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KDCTSGPYTAQIIPGTGNKLLMSSPNCEIYYQSPLSLCMAKRKSVSTP
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PAAQKAFQPPRSCGTKYETPIKKKELNSPQMTPFKKFNEISLLESNSIA
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LIKEQESSQASTEECEKNKQDTITTKKYI.

Figure 12



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Figure 13

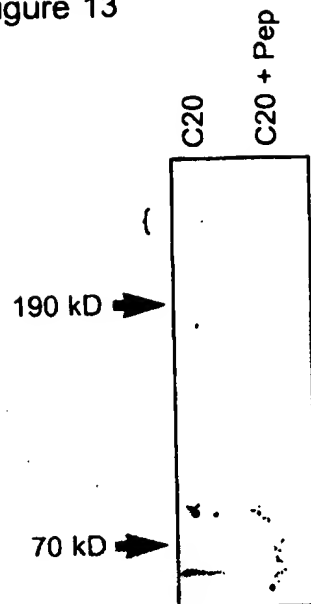


Figure 14

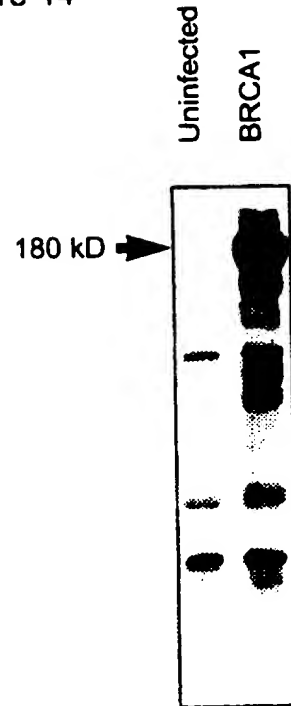
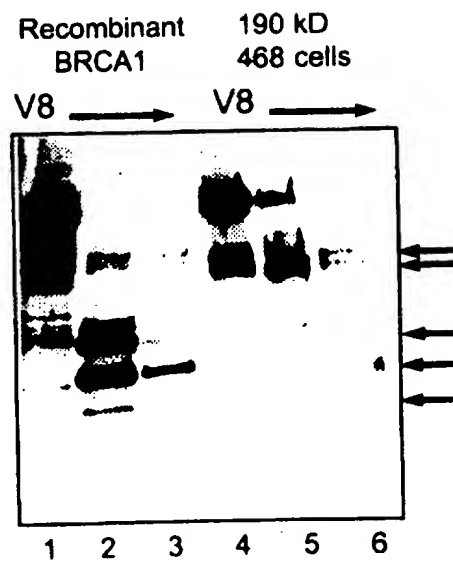


Figure 15



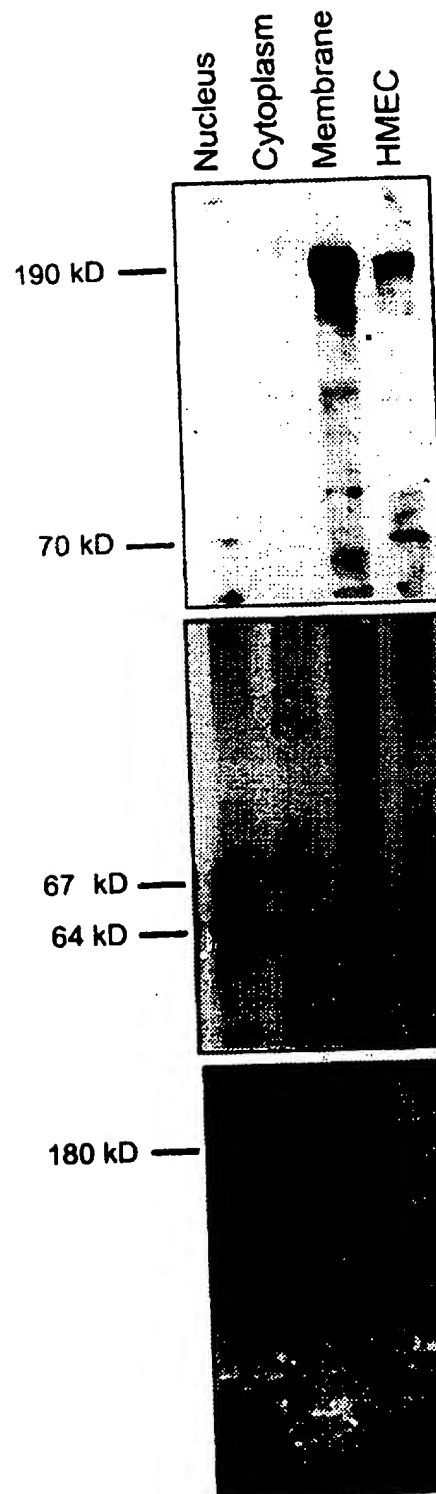


Figure 17

Figure 18

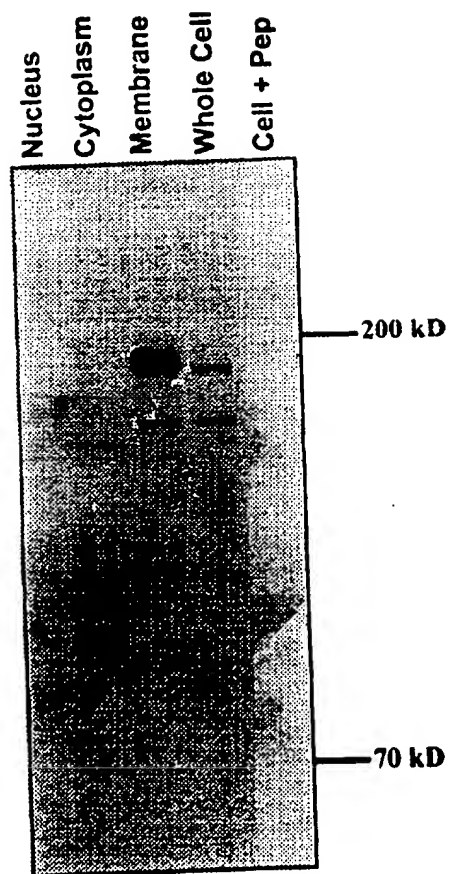
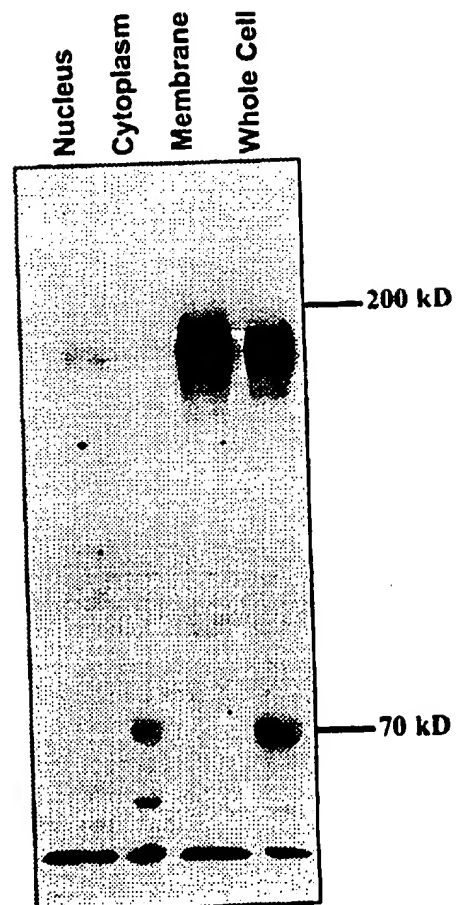
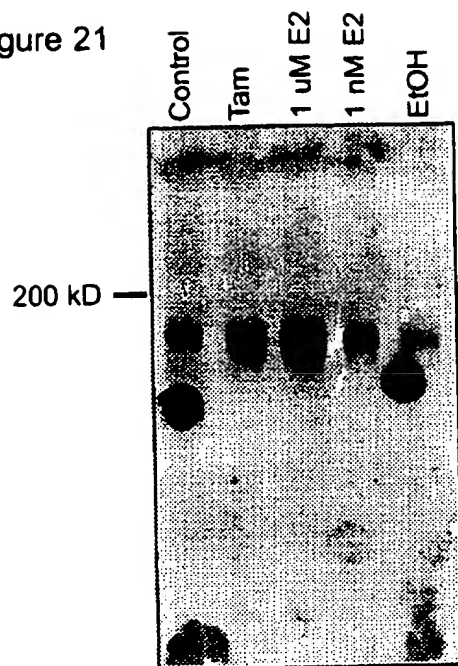


Figure 19

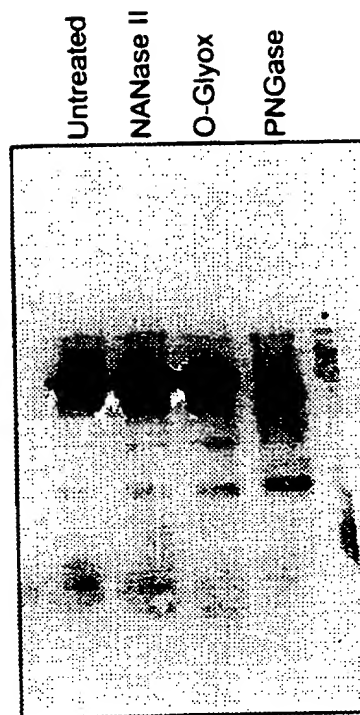


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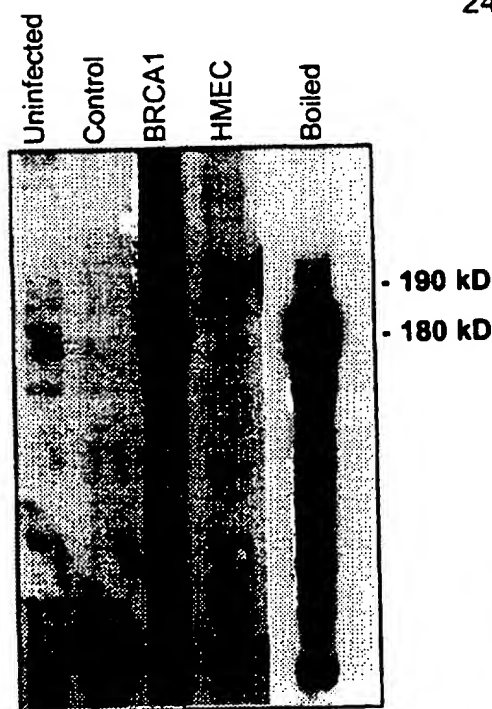
Figure 21



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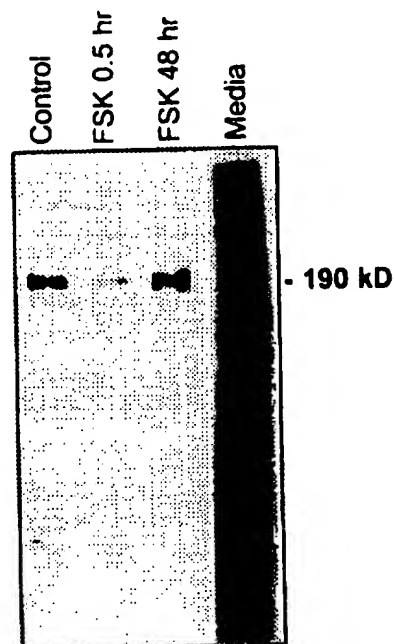


Figure 16

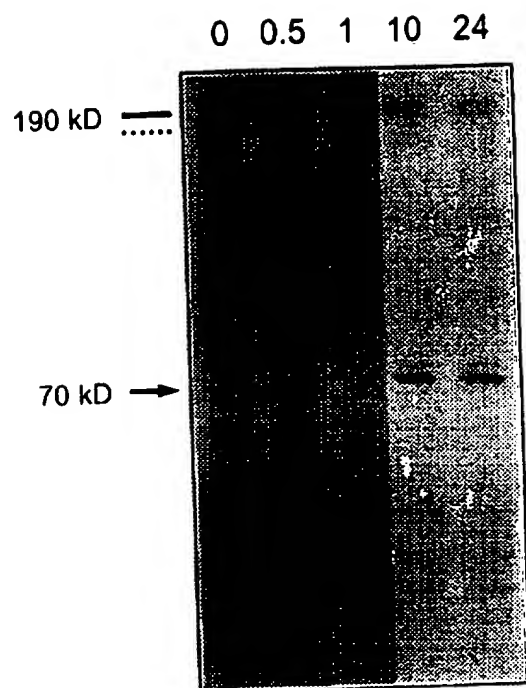
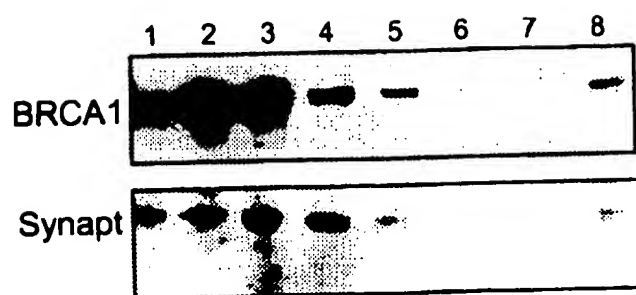


Figure 20



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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/03340

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C08H 1/00; G01N 33/566; C07K 1/00

US CL : 530/413; 436/501; 530/350

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/413; 436/501; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, CANCERLIT, BIOTECHDS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WOOSTER et al. Identification of the breast cancer susceptibility gene BRCA2. Nature. 21/28 December 1995, Vol. 378, pages 789-792, especially page 791.	1-7, 11, 40-43, and 50
Y	MIKI et al. A Strong Candidate for the Breast and Ovarian Cancer Susceptibility Gene BRCA1. Science. 07 October 1994, Vol. 266, pages 66-70, especially page 68.	1-7, 11, 40-43, and 50
Y, P	HOLT et al. Growth retardation and tumour inhibition by BRCA1. Nature Genetics. 12 March 1996, Vol. 12, pages 298-302, especially pages 301-302.	1-7, 11, 40-43, and 50
A	ORMISTON. Hereditary breast Cancer. European Journal of Cancer Care. 1996. Vol. 5, pages 13-20.	1-7, 11, 40-43, and 50

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 05 JUNE 1997	Date of mailing of the international search report 08 JUL 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVE NGUYEN Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/03340

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JONES et al. Molecular Genetics of Sporadic and Familial Breast Cancer. Cancer Surveys. 1995, Vol. 25, pages 315-334.	1-7, 11, 40-43, and 50
A	Proceedings of the American Association for Cancer Research. March 1996, Vol. 37, page 516, the Abstract No. 3532, ROMAGNOLO et al. Regulation of expression of BRCA-1 by estrogen in breast MCF-7 and ovarian BG-1 cancer cells.	1-7, 11, 40-43, and 50
Y	US 5,434,064 A (SCHLESSINGER ET AL.) 18 July 1995, columns 2-48, especially columns 2-6.	1-7, 50, 40-43, and 50
Y	US 4,675,285 (CLARK ET AL.) 23 June 1987, columns 2-10, especially columns 4-9.	1-7, 11, 40-43, and 50

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/03340

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-7, 11, 40-43, 12-17, 30-31, 50
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.